

Chapter 1

Introduction

1.1 Background

Nutrition refers to a scientific approach which studies the interaction occurring between living organism and food (Grosvenor & Smolin, 2002). Food provides essential nutrients and it is vital for the promotion of energy and maintenance of growth throughout life (Marchioni *et. al.*, 2005). It is also widely known that nutrition and health are closely linked and any form of malnutrition or over nutrition pose greater consequences of developing a disease. Of all the many diseases, cancers are of special importance due to the fact that it causes many premature adult deaths globally, after cardiovascular disease, and there have not been any miracle cure yet for treating this deadly disease (Taghavi & Yazdi, 2007).

Each of the food choices that an individual make usually contributes to the diet as a whole. In fact, the chosen diet must provide enough energy to fuel our body, essential nutrients to facilitate the process of our immune system and other food components with adequate amount to prevent deficiencies, promoting health as well as protecting against many other related chronic diseases. There is no single food choice which is good or bad within or of itself, but rather a combination of food choices that would make up a dietary pattern that is either healthy or not so healthy (Grosvenor & Smolin, 2002).

Traditionally, diet studies focused on the effects of certain nutrients or food items on the disease onset. However, in most developed countries, modification of certain food has been identified as a carcinogenic agent that would or most probably contribute to cancer (Shahar *et. al.*, 2004). In Malaysia, rapid socio-economic development has led many Malaysians practising unhealthy lifestyle, from consuming traditional diet pattern that is rich in complex carbohydrate, fruits and vegetables to a higher intake of fat and refined sugars. This is also complemented with advancement of technology in food processing methods and preservation that occurred simultaneously with one another that would possibly increases the incidence of many other chronic diseases including cancer.

Oral and oropharyngeal cancer are usually grouped together as oral cavity cancer with all the cancer sites grouped and termed as [ICD-10:C00-14] (International Classification of Disease 10th Revision) excluding salivary gland [C07-08] and other pharyngeal sites [C11-13] (Warnakulasuriya, 2009). Oral cavity cancer is ranked as the 11th most common type of cancer worldwide in terms number of cases and with over 390,000 new cases reported annually (Stewart & Kleihues, 2003), occurring mostly in developing countries (Filho, 2002). Meanwhile, oral cancer is predominantly a disease of old age; and was ranked among the top ten cancers in many parts of Asia which include countries of Southern Asia, Eastern, Western, Southern Europe, Australia and Melanesia (Parkin *et. al.*, 2001).

Oral cancer is also one of the emerging health problems in Malaysia. Report from the 2003 National Cancer Registry (NCR) in Malaysia, noted that oral cavity cancer incidence was ranked among the top twenty most common types of cancer ranging from the

gum, mouth, tongue and lip in both males and females respectively (Lim & Halimah, 2004). Based on the 2003 NCR data, incidence of oral cancer was ranked as 6th and 3rd highest among male and female ethnic Indians respectively while a hospital-based retrospective data on 5 states in Malaysia (Zain & Ghazali, 2001) indicates that the prevalence of oral cancer in indigenous people of Sabah and Sarawak was ranked the next highest. In a population-based nationwide survey on oral mucosa lesions carried out among 11,707 subjects in 1993/94, it was found that the prevalence for oral cancer was 0.4% and 1.4% for precancerous lesions (Zain *et. al.*, 1997). In terms of ethnic groups that make up the Malaysian populations, the Indians were found to have the highest prevalence with 4.0% followed by the indigenous people of Sabah and Sarawak with 2.5%, which were previously not known to be at risk (Zain *et. al.*, 1997).

It is widely known that tobacco smoking, alcohol consumption and betel quid chewing contributed to the risk of oral cancer (Macfarlane *et. al.*, 1995; Garavello *et. al.*, 2008; Amtha *et. al.*, 2009). However, several studies have also indicated that diet too plays an important role in cancer aetiology and prevention (Zain, 2001; Greenwald *et. al.*, 2001). According to the World Health Organization (WHO) reports, 30% of human cancers are attributed to dietary deficiencies or imbalances (Taghavi & Yazdi, 2007). Approximately 80% of the 250 cancer epidemiological studies undertaken, whether it is a case-control, cohort or ecological correlations, found a significant protective effect of overall consumption of vegetables and/or fruits (Stewart & Kleihues, 2003).

The association between dietary habits and risk of oral and pharyngeal cancer have been investigated in several Asian countries, both in relation to single food item or food

groups as well as to its nutrients, micronutrients or food components (Amtha *et. al.*, 2009; Lucenteforte *et. al.*, 2009). Dietary factors are also known to contribute to cancer prevention as past studies have shown a decreased risk with increasing consumption of Vitamins A and C, fresh fruits as well as green leafy vegetables (Vecchia *et. al.*, 1997; Zheng *et. al.*, 1993). Recent studies by different researchers also noted fruits (apples, pears and citrus fruits) as well as vegetables (raw or green leafy vegetables, tomatoes, and carrots) do offer beneficial health effects and reduce the risk of oral cancer (Lucenteforte *et. al.*, 2009). Generally, consumers should be aware on the benefits of consuming a healthy, well balanced diet that includes portions of fruits and vegetables daily that could help to minimise and prevent oral cancer risk.

1.2 Rationale of study

Oral cancer is a tobacco and quid-related disease that present significantly as a public health problem due to its relatively high incidence and prevalence in several parts of the world. The poor survival rate associated with this type of malignancy and the severe functional and cosmetics defects accompanying the treatment of this disease were the underlying reasons for numerous research studies. Oral malignancy can be prevented by finding better linkages to reduce the risk factors of oral cancer. It can also be cured if detected early by a combination of radiotherapy and surgical treatment. The American Institute for Cancer Research and the World Cancer Research Fund estimated that 30%-40% of all cancers can be prevented by appropriate dietary intake, physical activity and maintenance of body weight (Donaldson, 2004).

Although studies to determine the relationship between diet and oral cancer present a challenge due to food diversity or different food combinations, the basic assertion that dietary factors influence cancer risk is not a question for debate (Greenwald *et. al.*, 2001). However, many queries remain to be resolved, which include the specific dietary factors that are closely linked to cancer prevention, mechanisms on food components that exert putative effects, food interaction or competition that might affect cancer risk, bioavailability of certain nutrients in food and preventive measures that can be undertaken to reduce adverse effects on dietary factors that contribute directly to the disease risk (Greenwald *et. al.*, 2001; Marchioni *et. al.*, 2007). Thus, it is with great interest if future research on dietary relationship in terms of effects of certain nutrients or between food and food groups towards the risk of oral cancer would be explored fully.

Furthermore, research findings and reports from Western countries could not be fully applied to the local Malaysian scenario due to the complexity of food items consumed as well as their method of preparation which maybe quite different from that of the Western population. The dietary pattern and food intake may also be totally different from the Asian population due to food availability and cultural factors. Such complexity of food could also be due to the chemical content in food, while many food compounds could not be measured and are poorly characterized (Amtha *et. al.*, 2009). It was also found that the relationship is often attenuated after adjustment for potential confounding factors for oral cancer such as tobacco smoking and alcohol consumption. Therefore, this study is undertaken to establish the relationship between dietary intake and the occurrence of cancer in the oral cavity in the Malaysian population.

This study is expected to provide baseline information on the dietary pattern of the Malaysian population and also provide evidence to the hypothesized relationship between intakes of food and food groups with the risk of oral cancer among the people residing in this country. New knowledge generated from this study which uses the data from the Malaysian population on the relationship between the intake of certain food or nutrient will have a great impact to the country where nutritional intervention programmes and health education or awareness policies can be formulated by the relevant authorities. If dietary modification is found to be of significance especially in reducing the risk of cancer, it can also be used as one of the cheapest and easiest way of reducing oral cancer incidence in Malaysia through the existing health care delivery system.

With a carefully planned intervention programme based on sound scientific evidence and increased awareness through proper information dissemination and public education, a more beneficial effect will ensure. Thus, it will indirectly help in reducing the morbidity and mortality rates from not just oral cancers alone, but also from all other diet-related chronic diseases. Hence, in order to assess the dietary pattern of the Malaysian population, this research study will mainly focus on the following aim, specific objectives and hypothesis.

1.3 Aim

This study aims to assess the dietary intake of Malaysian, and to determine the association between dietary pattern and the risk of oral cancer in general. Additionally, it also aims to established risk indicators of micronutrients (β -carotene) intake with oral cancer.

1.4 Objectives

The three specific objective of this study are:

- 1) To identify the dietary pattern and β -carotene intake of oral cancer patients in a Malaysian population
- 2) To determine the association between consumption of food groups with regards to oral cancer risk
- 3) To determine the association between intake of β -carotene with the risk of oral cancer

1.5 Hypothesis

The null hypothesis of this study includes:

- 1) There is no association between consumption of food groups with oral cancer risk
- 2) There is no association between mean intake of β -carotene with the risk of oral cancer

Chapter 2

Literature Review

2.1 Definition of Oral Cancer

Oral cancer can be defined as malignant neoplasm that are caused by abnormal and uncontrolled cell division which involves the lip, tongue, mouth (oral cavity) [ICD-10: C00-06], oropharynx [ICD-10: C09-C10], excluding the salivary glands [ICD-10: C07-08] and other pharyngeal sites [ICD-10: C11-13] (Warnakulasuriya, 2009). These oral cancer sites were originally described based on the World Health Organization (WHO), International Classification of Disease 10th Revision (ICD-10) Version 2007. The most common form of oral malignancy (more than 90%) is squamous cell carcinoma (SCC) of the lining mucosa. The basic clinical features of oral cancer would be mucosal growth and ulceration, difficulty of speaking, opening the mouth and chewing, pain on swallowing as well as neck swelling (Sakagami, 2010).

2.1.1 Epidemiology of Oral Cancer

Oral cancer is a serious disease that presents significantly as a growing health problem in many parts of the world. Oral and pharyngeal cancers when grouped together represent the 6th most common cancer in the world (Toledo *et. al.*, 2010). Meanwhile, oral cavity cancers such as the pharynx and oesophagus, was estimated to account for almost 876,000 cases and 582,000 deaths in year 2000 (Key *et. al.*, 2004). Moreover, the incidence for oral cancers cases and mortality rate are on the rise with estimated new cases of 267,000

(2.7%) in year 2000 (Parkin *et. al.*, 2001). The areas characterized by high incidence of oral cancer cases are mostly found in New Zealand, Australia, Melanesia, followed by South East Asia which includes countries such as Sri Lanka, India, Pakistan and Taiwan (Filho, 2002 & Warnakulasuriya, 2009). Other pharyngeal cancers, apart from the countries mentioned above, are most commonly found in eastern, western and southern Europe (Pavia *et. al.*, 2006). Oral cancers are most common among males than females in high-risk countries such as Sri Lanka, India, Pakistan and Bangladesh where it contributes up to 25% of all new cases reported (Warnakulasuriya, 2009). Almost 95% of oral cancers were diagnosed in individuals over 40 years of age with the average age of diagnosis being 60 years (Boyd & Palmer, 2007).

In Peninsula Malaysia, the latest National Cancer Registry (NCR, 2006) reported that the age-standardized rate (ASR) of cancers was 128.6 per 100,000 in males and 135.7 per 100,000 in females respectively. The most common cancers in males were colorectal, lung, nasopharynx, prostate and liver while cancers of breast, colorectal, cervix, lung and ovary were predominant in females (Yip, 2010). Based on NCR (2006), oral cancers were recorded separately into three different segments that include the lip, mouth and tongue cancers. Among males, tongue and mouth cancers were ranked 21st and 26th while among females they were ranked 25th and 19th of all cancers respectively. However, when both mouth and tongue cancers were grouped together, cancer of the oral cavity (1.8%) was the 17th most common cancers among men and 15th most common cancers among females.

NCR (2006), further reported that the ASR for both mouth and tongue cancers increases with age. In terms of ethnicity and gender, Indians have a higher incidence of

tongue and mouth cancers than other races with an ASR of 8.1 per 100,000 and ASR of 13.9 per 100,000 respectively in the population. Furthermore, when all three cancers sites were accounted for, almost 7.1% of Indian males and 10.1% of Indian females had oral cancers, making it the 4th and the 2nd most common cancers among gender in Malaysia. Among Indian males, tongue and mouth cancers were ranked 6th (4.6%) and 14th (2.5%) of all cancers respectively (Omar *et. al.*, 2006). Among Indian females, incidence of mouth and tongue cancers were ranked 4th (7.3%) and 11th (2.4%) respectively. However, females had a higher percentage of lip cancer (0.2%) as compared to the males (0.1%).

Survival trends for oral cancers have shown little changes during the last few decades worldwide. Differences in survival rates among countries could possibly be due to the stages of disease diagnosis as well as the availability and quality of treatment (Filho, 2002). The five year survival rate of cancers of the tongue, oral cavity and oropharynx are around 50% for most of the countries as reported by Touger-Decker & Mobley, (2003) whereas a higher survival rate of over 90% are usually observed for cancer of the lip. For cancers of both tongue and oral cavity, women had higher survival rate than men (Warnakulasuriya, 2009). Generally, the prognosis decreases with advancement of the disease and increasing inaccessibility of the tumour site. In most oral cancers cases, survival rate is much higher for younger patients as compared to older patients.

2.1.2 Nutritional Epidemiology of Oral Cancer

Nutritional epidemiology investigates dietary or nutritional factors in relation to a disease occurrence in populations. Findings from nutritional epidemiology often contribute

to the evidence used in guiding dietary recommendations for prevention of cancers and other chronic diseases (McCullough & Giovannucci, 2006). Evidences from varied study designs are required to establish definitive relationship between diet and a specific disease since human diets are very complex. Many epidemiologists use population based approach to study the relationship between diet and cancer because randomized intervention trials are not always feasible, due to higher cost and it is ethically impossible to test a harmful exposure and cancer in humans. Therefore, the bulk of available evidence obtained for a particular diet-cancer relationship often uses observational nutritional epidemiological studies.

Recently, researchers have begun to assess dietary patterns in epidemiology because individuals eat foods in various combinations. This method can be tested for dietary combinations on disease prevention, using a variety of approaches such as principal component or factor analysis, the diet score method reflecting a hypothetically protective diet or dietary guidance (Hu *et. al.*, 1999). This diet-pattern approach usually characterizes foods or even for hypothesized interactive beneficial effects when these foods are consumed together. Dietary patterns could offer one way to test the effects of dietary combinations on disease prevention measure (McCullough & Giovannucci, 2006).

In analytical nutritional epidemiology, the most common form of studies are case-control, prospective cohort, and nested case-control (McCullough & Giovannucci, 2006). The information on diet and disease occurrence, in oral cancer study particularly, is collected on an individual basis and risk of disease is compared with those with no/low levels of exposure. For example in case-control studies, individuals who have been

diagnosed with oral cancer are interviewed with respect to their past diet and were matched on age or other pertinent factors. Results from these studies will be presented in the form of 'odds ratio' (OR) where the odds of developing a disease if given a certain dietary exposure is compared with the odds of developing a disease with no/low levels of dietary exposure. Prospective cohort studies of oral cancer are still lacking, and meta-analyses of other cancers, usually in the form of case-control studies may somehow overestimate some protective effect of a certain exposure (Maserejian *et. al.*, 2006).

2.1.3 Carcinogenesis of Oral Cancer

Carcinogenesis involves a multi-step process in which genetic events caused by Deoxyribonucleic acid (DNA)-reactive components, genotoxic carcinogens and growth promotion of mutated cells that signals a transformation of pathways governing normal cellular physiology which are quantitatively or qualitatively altered (Wong *et. al.*, 1996; Taghavi & Yazdi, 2007). When the body is exposed to carcinogenic agents, a variety of potential effects may occur and it does happen over a long period of time modulated by many factors (Boyd & Palmer, 2007). Some environmental factors or exposures such as ionizing radiation, smoking, specific infectious agents and dietary pattern may also be potentially harmful due to the accumulation of changes in the inhibitory and excitatory cells that may occur at any given pathways (Wong *et. al.*, 1996). The general overview of oral carcinogenesis process is illustrated in Figure 2.1.

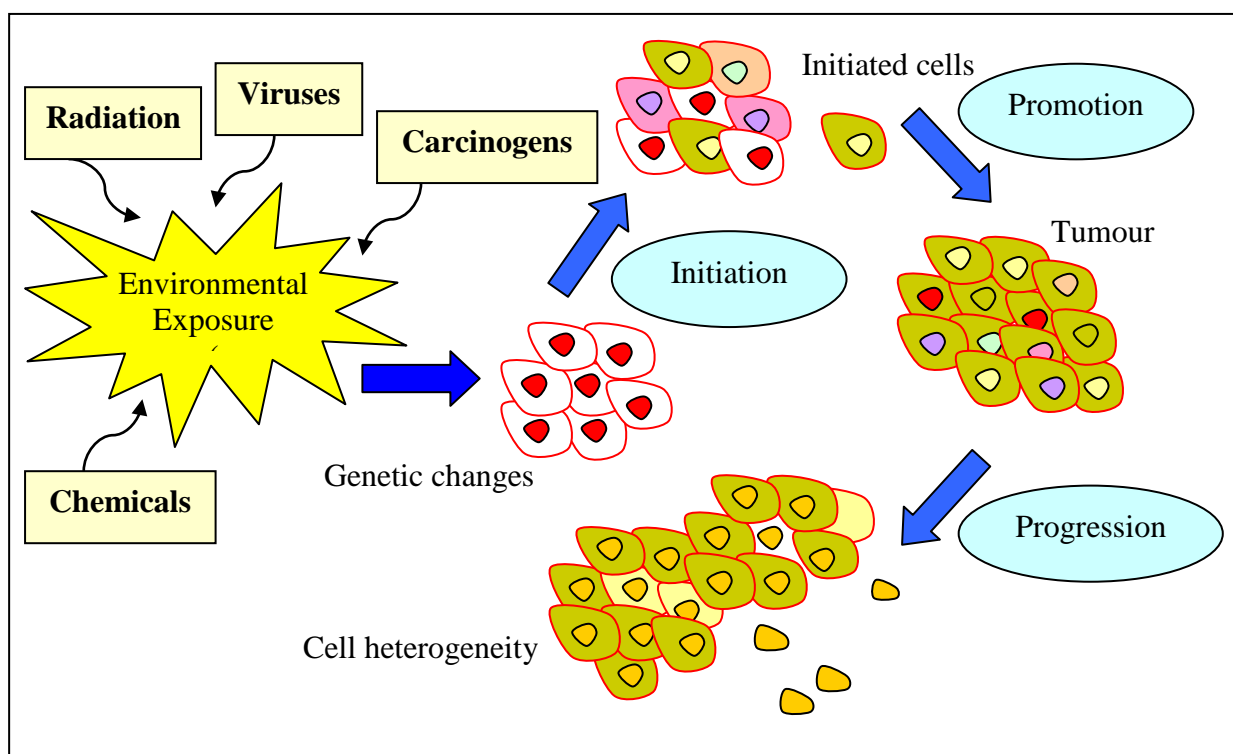


Figure 2.1 Multistep carcinogenesis: Initiations, Promotion and Progression stage

(Source: Amtha *et. al.*, 2009)

When the mutated cells continue to accumulate due to the cell's ability to proliferate at uncontrolled rate, inability to repair DNA damage within itself or refusal to self destruct/die (apoptosis), stimulate neo-vascularization, invading locally or metastasizing to distant sites, the cells would then become functionally independent from the surrounding oral epithelium (Wong *et. al.*, 1996). Numerous types of genes are likely to be involved in human carcinogenesis where these genes may influence the metabolic reactions/detoxification, DNA repair, chromosome stability, activity of oncogenes or tumour suppressor genes, cell cycle control, signal transduction, hormonal pathways, vitamin-metabolism pathways, immune function and receptor or neurotransmitter action (Greenwald *et. al.*, 2001).

2.1.3.1 Oral carcinogenesis in dietary pathway

Dietary intake does play a major role in oral carcinogenesis as many food components are involved in activating one or more steps of the cancer processes. Cancer progresses through several stages and it is only reasonable to assume that the same dietary components do not exert the same effect throughout the carcinogenic development (Kritchevsky, 2003). This could be due to the dietary chemicals that may alter the expression of one's genes or even the genomes itself (Heber & Bowerman, 2006). The relationship between nutrition and cancer can be approached at two different angles which are the direct or indirect effects (changes in metabolism due to alteration of dietary habits) of carcinogens present in food (Taghavi & Yazdi, 2007).

Numerous evidences showed that both essential and non-essential bioactive food component can interact with the genes and their expression in altering phenotypes (Milner, 2004). These genes will usually influence the absorption, metabolism or transportation of the bioactive food components, thus will alter the genetic expression of the host cellular, and eventually influences the cancer outcomes (Taghavi & Yazdi, 2007). It is also noted that the response to these bioactive food components are very much dependent on the number of cellular events and regulatory processes that will help to maintain a balanced state of mankind and/or survival (Milner, 2004). Dietary carcinogens such as polycyclic aromatic hydrocarbons (PAHs) can alter DNA by forming adducts. Recent studies also reported that a diet rich in fruits, vegetables and cereals are associated with the reduction of white blood cell PAH-DNA adducts, as well as consumption of carotenoid-rich food

sources such as carrots and tomatoes reduced oxidative DNA damage in human lymphocytes by various mechanisms (Greenwald *et. al.*, 2001).

Some cruciferous vegetables, such as broccoli, cauliflower, brussels sprouts and cabbage have unique substances of dithiolthiones and isothiocyanates (organosulphur compounds) where studies have shown that they cause an increase of enzyme activities involved (by inducing phase I and II) in the detoxification of carcinogens (Steinmetz & Potter, 1996). In citrus fruit, a high content of Vitamin C may protect the cell membrane and DNA from oxidative damage. Vitamin C may further prevent cancer via its ability to reduce nitrate and subsequently the formation of nitrosamine (Smith-Warner *et. al.*, 2006). Green leafy vegetables, yellow/orange fruits and vegetables contain significant amounts of lutein, β -carotene and xanthophyll pigments that may protect the damage of free radicals in our body. Beta-carotene can also be metabolized into Vitamin A (retinol) which may further inhibit cell proliferation as well as inducing cell-to-cell communication (Steinmetz & Potter, 1996).

2.2 Risk Factors of Oral Cancer

Risk factors for oral cancer and pre-cancer are considered to be multifactorial (Warnakulasuriya, 2009). However, the more important factors, which are well documented by several epidemiological studies, are still the association between the habit of betel quid chewing, smoking and alcohol consumption with oral cancer (Amtha *et. al.*, 2009; Zain, 2001; Warnakulasuriya, 2009). The risk factors of oral cancer are often associated with

tobacco smoking and alcohol consumption that attribute up to 90% in many developed countries (Filho, 2002; Prasad *et. al.*, 1995).

In Western countries, many researchers have documented that smoking and alcohol consumption does play an important role in oral carcinogenesis (Kumar & Zain, 2004; Vecchia *et. al.*, 1997; Macfarlane *et. al.*, 1995; Filho, 2002), where these associations were found to show a dose response relationship. The combination of alcohol consumption and tobacco smoking synergistically increases the risk and development of premalignant lesions (Khaodhiar & Blackburn, 2006 and Johnson, 2001). In most oral cancer cases, studies have also noted that individuals who smoke regularly will also tend to drink regularly (Gerson, 1990). Greenwald *et. al.* (2001) in a review study noted that consumption of ≥ 150 ml ethanol/day and smoking ≥ 25 cigarettes/day, both independently increased the risk of oesophageal cancer. The evidence of interaction between cigarette and alcohol use in both male and female was associated with greater risk of multiplicative effect for this combined exposure (Zavras *et. al.*, 2001). Therefore, in most of the epidemiologic studies reviewed, researchers have concluded that consumption of alcoholic beverages or the use of tobacco either alone or in combination may cause cancer.

Besides the main habitual factors mentioned above, studies on the implications of molecular or genetic alterations, human papillomavirus (HPV), poor oral health, mouth wash and smokeless tobacco to oral cancer risk have also been investigated over the last decade (Filho, 2002; Gillison *et. al.*, 2000; Prime *et. al.*, 2001; Vecchia, *et. al.*, 1997). However, data on the role of other potential factors such as dietary intake is still lacking and has not been properly explored.

2.2.1 Betel Quid Chewing

Betel quid chewing is still widely practised and considered the most common habits among the Southeast Asia countries especially in India, Pakistan and Taiwan (Kumar & Zain, 2004 and Pavia *et. al.*, 2006). Whilst the major ingredients are similar, preparation may vary across different countries. A distinctive difference could be observed where tobacco was added as an ingredient by betel quid chewers from India and South East Asia but are rarely used in Taiwan (Lee *et. al.*, 2003). In India, a nested case control study indicated that tobacco quid chewing was the strongest risk factor associated with oral cancer and the adjusted OR for the chewers in men were 3.1 and 11.0 for women (Muwonge *et. al.*, 2008). This finding is in accordance with an earlier study done by Dikshit & Kanhere, (2000) where similar findings of about six-fold increased risk was observed. Meanwhile, a cohort study conducted in Taiwan also indicated that an increased risk of almost 10–fold in developing oral cancer for those who chew betel quid (Yen *et. al.*, 2008).

In Malaysia, it is noted that two ethnic groups (Indians and Indigenous people) share a common established risk habits for oral cancer which is habitual quid chewing. In a prevalence study conducted by Tan *et. al.*, (2000) in Peninsula Malaysia, it was reported that more than 19% of the estate population still practised betel quid chewing. The habits of quid chewing are mainly found among the elderly Malays in non-urban areas, the Indians in estate plantations, the Orang Asli communities and the Indigenous people of Sabah and Sarawak (Zain & Ghazali, 2001). Figure 2.2 shows the main ingredients usually used by betel quid chewers which are areca nut (seed of *Areca catechu*), betel leaf (leaf of *Piper*

betel) and slaked lime (boiled from seashells). The indigenous people of Sabah and Sarawak will add tobacco and gambir (a preparation from leaves and twigs of shrub *Uncaria gambir*) to the quid mixtures. The Indians will add tobacco into the quid while the Malay quid chewers do not include any into the quid mixtures (Zain & Ghazali, 2001). Quid chewing is not practised by the Chinese populations, but a dying habit for the Malays and Indians communities.



Figure 2.2 Ingredients commonly used by quid chewers in Malaysia

2.2.2 Alcohol consumption

Many epidemiological studies strongly support a direct relationship between alcohol consumption and oral cancer risk (Levi, 1999). Consumption of beer, wine, spirits and total alcohol beverages are strongly associated with increased risk of oral cancer (Chyou *et. al.*,

1995). This is consistent with an earlier case-control study conducted in India that noted a relative risk of 1.42 with a dose response relationship in terms of frequency and duration of the habit (Rao *et. al.*, 1994). Similar risk was also observed in a recent study from Italy which found an odds ratio of 2.1 among males who consumed an excess of 120g alcohol per day (Llewellyn *et. al.*, 2004). On the contrary, a study in Greece by Zavras *et. al.*, (2001), found no significant association with increase consumption of alcohol per week ($P_{\text{trend}} = 0.07$).

The mechanisms and effects of alcohol on the cancers are not widely known but it may involve direct contact on the epithelial cells (Key *et. al.*, 2004). Alcohol acts as a solvent, thus facilitating the passage of carcinogens through cellular membranes (Vecchia *et. al.*, 1997). Some of the possible mechanism that could increase the overall cancer risk includes carcinogenicity of acetaldehyde (a metabolite of alcohol) in alcoholic beverages, effects on cell membrane, effects on carcinogen metabolism, alteration of hormone levels or even the impairment of nutrient metabolism.

In Malaysia, many ethnic groups, especially the Chinese and the Indians, usually consume beer and stout on festive occasions. Special homemade brands such as toddy (tapped from the coconut palm, with varying degree of fermentation) and samsu (locally brewed Chinese alcohol) are also consumed by the Indians and Chinese as well as domestically manufactured rice wine alcohol or known locally as *tapai*, used mostly by the indigenous people of Sabah and Sarawak (Zain & Ghazali, 2001). So far, there has not been any prevalence studies reported in Malaysia on the total consumption or intake of alcoholic beverages among ethnic groups with the risks of oral cancer.

2.2.3 Tobacco smoking

Tobacco is consumed in many different ways, but smoking of manufactured cigarettes is one of the most common forms. Past research studies have noted that smoking tobacco either in the form of cigarettes, cigars or pipes may lead to oral cancer. The content of cigarettes varies greatly with the amount of tar, nicotine and nitrosamine, as well as depending on the species, curing additives and method of combustion (Johnson, 2001). Generally, most of the case-control studies have reported a dose-risk relation for tobacco smoking, where increasing the consumption lead to higher risks of oral cancer (Gerson, 1990 and Macfarlane *et. al.*, 1995).

Smoking is estimated to be the main cause of cancer with almost 40-45% of reported deaths worldwide. Studies conducted in the European continent also reported a modest risk for oral tongue cancers with odds ratio of 1.8 for smokers using 16 to 25 cigarettes per day and increasing to odds ratio of 2.1 for smokers using 35 or more cigarettes per day (Llewellyn *et. al.*, 2004). A similar study conducted in Spain also noted that the odds ratio (OR) for developing oral cancer for those who smoke 6-20 cigarettes/day was 3.1 which increased to 8.0 among those who smoke more than 20 cigarettes/day (Moreno-Lopez *et. al.*, 2000). The overall risk of oral cancer among smokers was usually 7-10 times higher than those who never smoke (Warnakulasuriya *et. al.*, 2005)

In Malaysia, the types of tobacco smoked includes commercial brand cigarettes such as 'bidi' which is commonly used by Indians, hand-made (special paper-rolled cigarettes) or locally known as 'rokok kertas', hand-made (Nipah leaves-rolled cigarettes)

or locally known as 'rokok daun' and 'kretek' (an Indonesian imported type of cigarettes that contains other ingredients such as cloves) (Zain & Ghazali, 2001). In an earlier research compiled by Ramanathan & Lakshimi (1976) with regards to oral carcinoma, their findings concluded that the most common oral habit among the three main ethnic groups in Malaysia, were still the habits of cigarettes smoking.

2.2.4 Dietary intake

Numerous researchers have investigated the relationship between dietary intakes with the occurrence of cancer. Most of the studies with regards to dietary intake and oral cancer were carried out in European populations especially among the Italians (Garavello *et. al.*, 2008 and Lucenteforte *et. al.*, 2009). Studies in the Asian region have been done among the Indonesian population (Amtha *et. al.*, 2009), the Indians (Rajkumar *et. al.*, 2003; Prasad *et. al.*, 1995), the Chinese (Zheng *et. al.*, 1993) and the Japanese communities (Takezaki *et. al.*, 1996). The type of food items studied varied widely from one study to the next with the most common type of food studied in most of the research was the intakes of fruits and vegetables.

The evidence of the protective effects of consuming high intake of vegetables and fruits is consistent with the 206 reviews of epidemiologic and 22 animal studies which were summarized by Steinmetz and Potter (1996). Many epidemiological studies have also indicated that consumption of fruits and vegetables have been associated with lower risk of lung, oral, oesophageal, stomach and colon cancer (Sapkota *et. al.*, 2008; Steinmetz and Potter, 1996; Stefani *et. al.*, 1999a). Earlier studies conducted by Levi *et. al.* (1998), also

noted a significant reduced risk of oral and pharyngeal cancers with odds ratio of 0.24 in vegetables and 0.34 in fruits respectively. The International Agency for Research on Cancer (IARC) also reported similar findings in a multinational case-control study with a significant reduced risk of oral cancer among individuals with high intake of fruits and vegetables (Kreimer *et. al.*, 2006).

Dietary studies carried out in Western countries with high oral cancer incidence rate especially from the European continent have shown that high intake of fat food items such as meat and low intake of fruits and vegetables seems to confer an increased risk for oral cancer (Greenwald *et. al.*, 2001; Key *et. al.*, 2004; Donaldson, 2004; Garavello *et. al.*, 2009; Lucenteforte *et. al.*, 2008; Stefani *et. al.*, 1999a). Besides that, deficiencies of Vitamin A, E, C, B₁₂, folate and β -carotene may potentially increase the risk of oral precancerous lesions. Similarly, high dietary intake of folate may help to reduce risk of oral and pharyngeal cancers (Boyd & Palmer, 2007). The validity of these findings still remains doubtful as the confounding effects have not been adequately controlled for.

A multivariate study among 282 histologically confirmed cases of nasopharyngeal cancers (NPC) among Malaysian Chinese by Armstrong *et. al.*, (1998) found a strong positive association between intake or consumption of salted preserved foods (fish, leafy vegetables, eggs, roots, fresh pork / beef, organ meats) and NPC while vegetables and fruits combinations were found to have strong negative association with NPC. A study on the association between fibre intake and risk of cancer on 100 pairs of cancer cases and healthy controls was conducted by Shahar *et. al.*, (2004) where it was found that the percentage of energy contribution from fat was high among cases (35%) as compared to controls (32.1%)

while the mean dietary fibre intake was noted to be lower in cases (10.9 ± 8.9 g/day) than controls (13.2 ± 6.0 g/day) with significant difference noted for those who had breast and NPC. So far, in Malaysia, there is no study that has been reported on the relationship between dietary intakes and risk of oral cancer.

2.3 Dietary pattern and risk of oral cancer

Diet is a pattern of an individual food intake that consists of eating habit as well as the kinds or amounts of food eaten. Diet as a whole maybe affected by a host of psychosocial factors such as ethnic background, tradition, religion, lifestyle peer influence, personal attitudes and health condition (Palmer & DePaola, 2007). A healthy diet usually contains all the necessary nutrients in adequate amounts to meet daily requirements. In fact, there is no 'good' or 'bad' food specifically, but planning an adequate diet requires making wise food choices that includes a variety and a moderation of foods consumed to maintain health and prevent chronic diseases such as cancer.

Studies on diet and cancer are highly complex as there are no concrete studies which indicate how single or combination of nutrients, energy imbalances and the distributions of body fat that could predispose an individual towards developing specific cancers (Byers *et. al.*, 2002). Diet is made up of numerous foods with varied nutrients that are prepared in many ways to contribute energy for the body in support of basic cellular needs (Heber & Bowerman, 2006). Foods are made up of basic macronutrients such as protein, carbohydrate and fat that plays a major role in determining the impact of dietary patterns towards the risk of cancer. Somehow, what is of concern is not the food intake but

the marked differences in how each food items/groups are digested or absorbed and metabolized in our body for functionality.

Researchers are beginning to assess dietary patterns in epidemiology because individuals eat foods in various combinations and manipulation of a single nutrient usually affects several dietary exposures. Moreover, the assumption that single food items or nutrients may have isolated effects could not be valid due to the combination of joint effects between foods and nutrients that are likely to interact synergistically (Flood *et. al.*, 2008). For the purpose of this research, a diet-pattern approach was applied, in the hope of capturing the totality of dietary experience including all food or nutrients interaction among the population in Malaysia.

Dietary pattern in oral cancer studies from developing countries such as in Brazil found that “prudent” and “traditional” patterns consists of high intakes of fruits, vegetables, rice and pulses was inversely associated with oral cancer with an odds ratio of 0.44 (Toledo *et. al.*, 2010). This is consistent with an earlier study conducted by Marchioni *et. al.*, (2007) where an inverse association with oral cancer risk was found for “traditional” pattern but a positive association was noted for ‘monotonous’ patterns respectively. Similar studies on nutrient-based dietary patterns conducted in Italy provided information on “animal products” pattern that was positively associated with oral cancer where the odds ratio of 1.56 was observed (Edefonti *et. al.*, 2010). The only Asian study by Amtha *et. al.*, (2009) which was on dietary pattern consumption showed that ‘preferred’, ‘chemical-related’ and ‘traditional’ patterns have about twice the risk of oral cancer with adjusted odds ratio of 2.17, 2.56 and 2.04 respectively.

It is also interesting to note that dietary pattern studies on other cancer sites have also been conducted such as colorectal cancer among Americans where dietary pattern consisting of high intake of red meat and potatoes were associated with increased relative risk of 1.17 for men and 1.48 for women (Flood *et. al.*, 2008). Dietary pattern study was also conducted for lung cancer among Uruguay men where similar increased risk was also observed for consumption of ‘high-meat’ pattern with an odds ratio of 2.9 (Stefani *et. al.*, 2008). On the other hand, consumption of ‘prudent’ pattern in Japanese women was found to be negatively associated with breast cancer risk of almost 27% (Hirose *et. al.*, 2007).

2.4 Basic need of human nutrition

Diets are made up of numerous foods in varied proportion that are prepared in many different ways (Donaldson, 2004). Foods provide basic cellular energy needs which are made up of the basic macronutrients such as protein, carbohydrate and fats. They play a major role in determining the impact of dietary patterns on the risk of cancer (Heber & Bowerman, 2006). Within each category of macronutrient, there are marked differences on how these food sources are digested, absorbed and metabolized by the body to provide substantial health benefits to an individual (Murano, 2003).

2.4.1 Carbohydrate

Carbohydrates are also known as a class of nutrients that ranges from simple sugars like (glucose, fructose and galactose) to complex carbohydrate such as (starch, cellulose) and indigestible fibers. The basic unit of carbohydrate (single sugar molecule) is also

known as monosaccharides. Figure 2.3 show some of the main monosaccharide carbon structure. However, when two sugar molecules combine, they form disaccharides. For more complex carbohydrate called polysaccharide, it is made up of many monosaccharides with the longer sugar molecules being linked (Grosvenor & Smolin, 2002).

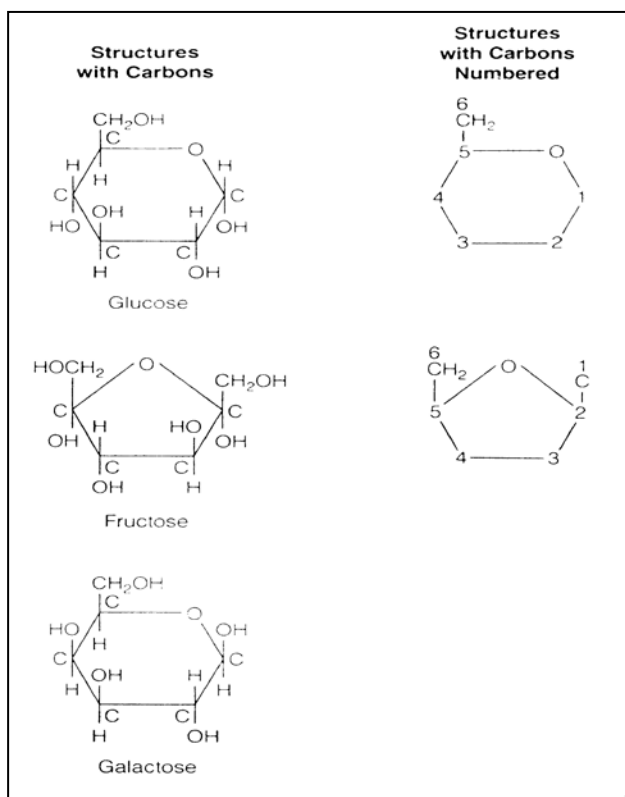


Figure 2.3 Chemical structures of monosaccharides with attached carbons

(Source: Illustration adapted from Murano, 2003)

In the diet, carbohydrate is usually found in grains, breads, legumes, fruit, vegetables, honey and sugar. The primary function of carbohydrate is to provide a source of energy to facilitate body metabolism and control of body temperature (Weill & Boyd, 2007). A constant supply of glucose is delivered to cells via the bloodstream to fuel the

body with enough calories for daily work. A dietary pattern that consists of high unrefined carbohydrates (especially whole grains, vegetables and fruits) has been associated with a lower incidence of a variety of chronic diseases which includes colon cancer, heart disease and diabetes (Donaldson, 2004). For example, consuming foods high in fiber affects the intestinal microflora and the by-products of microbial metabolism, thus decreases the contact time between the mucosal cells and probably the development of cancer (Grosvenor & Smolin, 2002).

2.4.2 Protein

Proteins are needed by the body for cell growth, repair and maintenance of tissue as well as serving numerous functions in the body namely, regulating enzymes, antibodies, hormones and acid-base balance (Heber & Bowerman, 2006). Protein works as an integral part of our human body structures that includes formation of skin, muscles and bones. Protein is made up of amino acid which is derived from both amino (NH_2) group and acid (COOH) group attached to the central carbon of the molecule. The protein molecule structures of different functional amino acids are presented in Figure 2.4

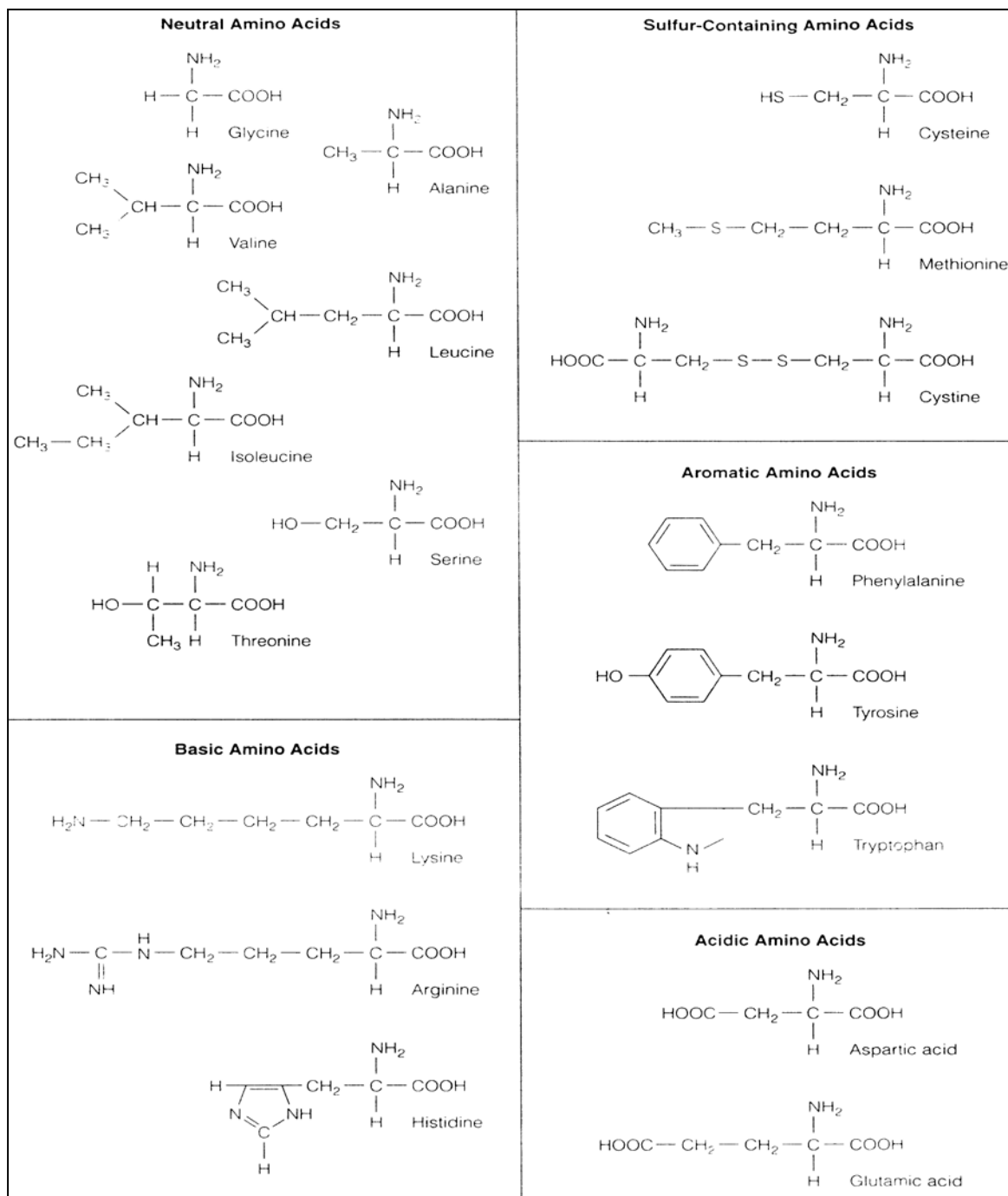


Figure: 2.4 Different types and functions of basic chemical structure for amino acids
(Source: Illustration adapted from Grosvenor and Smolin, 2002)

There are 20 amino acids found in the body. Eleven of these (alanine, arginine, asparagine, aspartate, cystine, tyrosine, glutamate, glutamine, glycine, proline and serine) can be synthesized in our body and are therefore known as non essential amino acids (Grosvenor & Smolin, 2002). The remaining 9 amino acids (histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine) cannot be synthesized in the body or are not made in sufficient amount to meet the body requirements and are therefore termed as essential amino acids (Heber & Bowerman, 2006).

Foods that contain essential amino acids are mostly supplied in the diet from animal sources such as meat, dairy milk or from soy proteins such as beans and legumes. Some epidemiological studies suggest strong positive correlations between protein intake and incidence of cancer but evidence are dependent on the source and amount of foods consumed (McIntosh & Le Leu, 2001). Therefore, a proper balance and sufficient intake of amino acids is important for production of essential amino acids to ensure good protein quality is achieved.

2.4.3 Lipids, Fats and Fatty Acids

Lipids (fats) are a group of organic compounds, most of which does not dissolve in water and include fatty acids, triglycerides (fats and oils), phospholipids and sterols (Boyd & Palmer, 2007). Fatty acids are also denoted by the length of the carbon chain and the number of double bonds they contain in dietary fats which is illustrated in Figure 2.5. Fat plays an important role in energy balance by enabling efficient fuel storage of calories in adipose tissue. Our body is capable of synthesizing saturated fats from carbohydrates but

the polyunsaturated essential fats must be taken from the diet. The degree of saturation influences the texture of fats, where most polyunsaturated fatty acids (vegetable oils) are mostly in liquid form at room temperature as compared to the saturated fatty acids (animal fats) which are usually solid in nature (Heber & Bowermann, 2006).

Lipids occur naturally in foods such as meats, dairy products, poultry, fish, nuts, vegetable oils and are added in most food processing and preparation. Some fatty acids such as Omega-6 and Omega-3 must be consumed from the diet as human are not able to synthesize them. These fatty acids are useful for anti-inflammatory, growth, fertility, cell structure and functionality of the central nervous system. Contradictory findings were noted as consumption of Omega-6 appears to enhance the promotional phase of carcinogenesis for breast, colon and prostate cancer, whereas Omega-3 seems to exert inhibitory effects (Greenwald *et. al.*, 2001). Overall findings also suggest that the link between fat and cancer risk depends on the type of fat consumed rather than or in addition to the total fat intake (Grosvenor & Smolin, 2002).

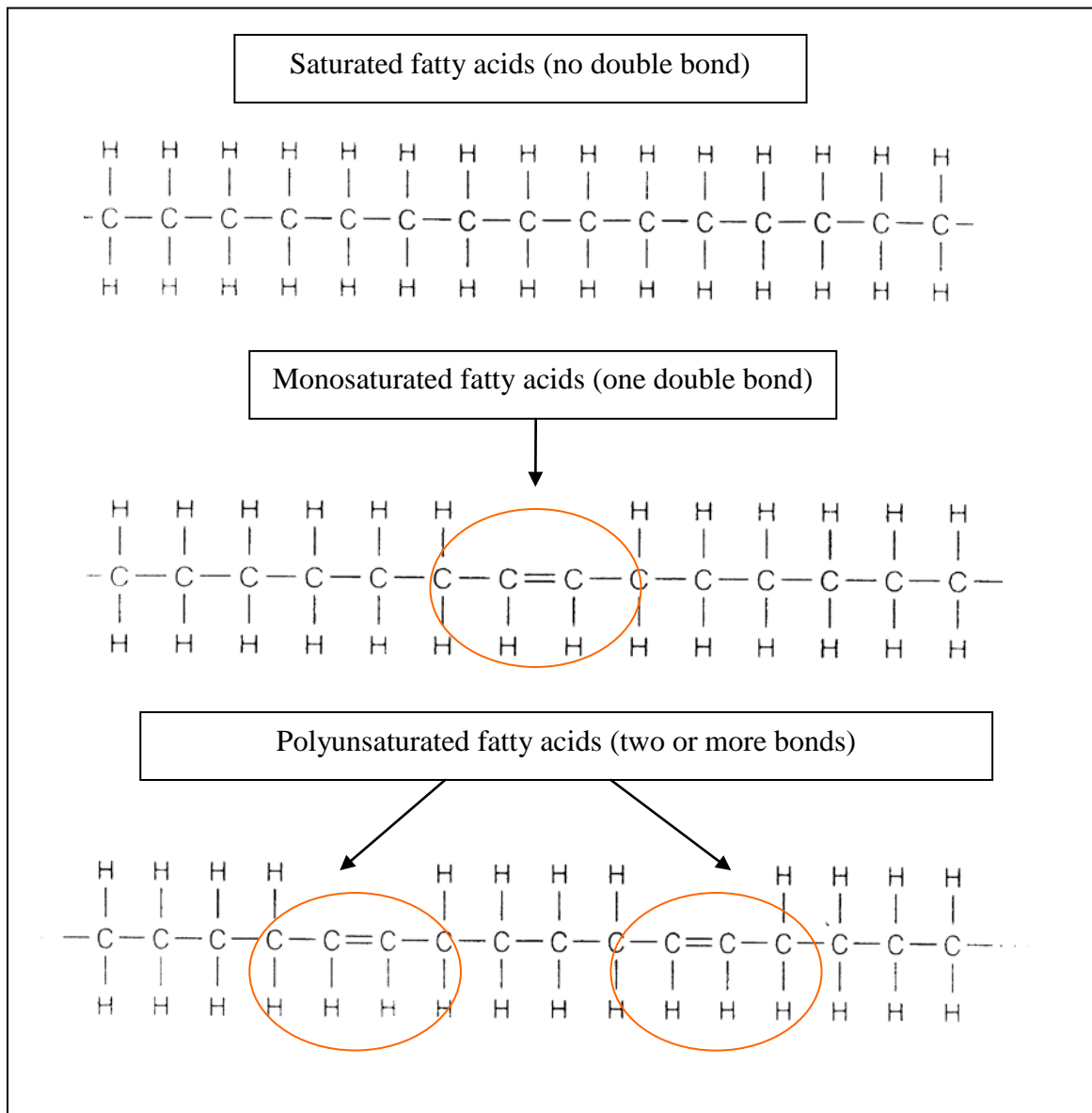


Figure 2.5 Fatty acids structure denoted by length of carbon chain and number of double bonds (Source: Illustration adapted from Murano, 2003)

2.5 Nutritional Assessment

Good nutrition is critical for the general well being of the society at large. Likewise, the increased life expectancy, higher living standards, sedentary lifestyle, environmental stress and abundance of food have exposed individuals to different types of chronic diseases. Therefore, to effectively enhance their health status through improved nutrition, nutritional assessment is being used as an important tool by health care professionals. Nutritional assessment can be defined as ‘an evaluation of the nutritional status of individuals or populations through measurement of food and nutrient intake, thus to identify possible occurrence, nature and extent of impaired nutritional status’ (Lee & Nieman, 1996). In order to identify each individual nutrient status, measurement can be accessed via four different methods such as anthropometric, biochemical, clinical and dietary.

2.5.1 Anthropometric Method

Anthropometric measurement involves determination of physical dimensions and gross composition of the body site. This method includes measurement obtained from weight, height, and head circumference as well as skinfold thickness to estimate percentage of fat and lean tissue in the body. Body Mass Index (BMI) and Waist to Hip Ratio (WHR) are other important measurement tools to identify and collect information in designing appropriate nutrition intervention programmes. The measurements are often compared to standard values obtained from measurement of large numbers of study sample (Lee & Nieman, 1996).

2.5.2 Biochemical Method

Biochemical method relies on the examination of metabolite in blood, faeces, urine or tissue samples to assess nutrient intake. Some examples of these methods involve quantity of serum protein or albumin as an indicator of body's protein status, haemoglobin level in blood that reflects iron status and blood cholesterol level for detection of any heart disease risk. Typically, a complete blood count is very useful to assess nutritional status of an individual such as micronutrients contents in order to determine potential problems and maintain overall wellness in an individual.

2.5.3 Clinical Method

Clinical methods refers to medical history, complete physical examination, blood pressure measurements, family history or stress factor of a person to detect signs or symptoms of a disease onset. Signs and observations were first made by qualified examiners during physical examination to observe any visible clinical signs. Medical records of an individual in the clinical setting are relevant and may be useful to support and reinforce a suspected diagnosis of any nutritional deficiency.

2.5.4 Dietary Method

Dietary assessment is used to identify and quantify types and amount of foods and beverages consumed by an individual. An ideal dietary assessment should provide an accurate, objective, unbiased and quantitative measure of long term exposure to the dietary

constituents (Carpenter, 2006). Dietary assessment usually involves surveys that measure the quantity of food consumed by the individual during the course of one to several days or assessing the pattern of food intake during the previous months. These can provide data on intake of nutrients or specific classes of foods. There are several identified dietary assessment methods which include twenty-four hour dietary recall, food records or food frequency questionnaire (FFQ). Many different types of nutrient composition database software programs such as NutriCal or NutrieMart (Thompson & Subar, 2008) can be used to convert dietary data and thereafter to analyze the nutrient intake of an individual.

2.5.4.1 Food Record/Diary

Food record/diary is basically a detailed description of the types or amount of foods and beverages consumed over a presumed period, usually about 3 to 7 days (Grosvenor & Smolin, 2002). Food records may come in the form of a diary or booklet where subjects are required to write down all foods and drinks they consumed throughout the stipulated period. Generally, dietary record is inexpensive but inappropriate to assess past intake. It requires meticulous record keeping but is very useful for immediate alterations of a diet plan when the respondents are much aware on what they were eating.

2.5.4.2 Twenty-four Hour Diet Recall

Twenty-four hour diet recalls generally ask respondents to describe the foods and beverages consumed over the past 24 hours. Usually, this method will be conducted via face to face interview or telephone interview with a trained personnel/nutritionist for

dietary data collection. Twenty-four hour diet recalls are highly accurate and a reliable measure for recent intake but it does not reflect the whole variability of food consumed by the respondent. It is not recommended for large-scale cohort studies (Carpenter, 2006).

2.5.4.3 Food Frequency Questionnaire (FFQ)

Many dietary studies involving FFQ are commonly used to rank or group study subjects for the purpose of assessing the association between dietary intake and disease risk such as in case-control or cohort studies (Thompson & Subar, 2008). FFQ are specifically designed to assess frequencies with which food items are usually consumed during a specified time. FFQ generally provide a listing of food that includes columns to mark, indicating how often the food is consumed on a monthly, weekly or daily basis. This approach is important to measure consumption exposure over a period of time when it also reliably estimates the effects of foods during the 20-30 year cancer induction period. Most epidemiological studies have used FFQ extensively in terms of finding the association between diet and risk of cancer (Carpenter, 2006).

FFQ are relatively inexpensive, easy and quick to administer which is highly relevant for health survey purposes. A pre-defined food list will be adapted according to the population of interest and mimic the actual food habits. FFQ estimates the effects of health outcomes with regard to diet and are most frequently quantified as odds ratio or relative risks in many epidemiological studies (Haftenberger *et. al.*, 2010). Although recalling a diet for the past 20 years or so is considered impractical, this approach may approximately

determine the habitual dietary patterns that were present over the long term (Carpenter, 2006).

2.6 Phytochemicals constituents in food

Foods contain a wide range of vitamins and minerals, phytochemicals, estrogenic compounds, chemicals and natural pesticides, microbial toxins and chemicals formed during cooking (McCullough & Giovannucci, 2006). Fruits, vegetables and whole grains contain thousands of phytochemicals. Phytochemicals are biologically active substances in plants that provide colour, odour, flavour, and defence mechanisms (Palmer *et. al.*, 2007). The term functional food indicates this presence of bioactive substances that may affect physiologic benefits beyond basic nutrition (McCullough & Giovannucci, 2006 and Grosvenor & Smolin, 2002).

Consumptions of plant foods (e.g. fruits and vegetables) will provide millions of phytochemicals and non-nutritive substances that may provide health protective effects. These phytochemicals can be divided into different types of substance that are present in the plant itself such as allyl sulfides in garlic and onions; phytates in grains; lignans in flax, isoflavones in soybeans; isothiocyanates in cruciferous vegetables; and a whole range of flavonoids, carotenoids and terpenoids in various fruits and vegetables (Craig, 1997). Apart from this, the most documented and well researched studies on vitamin/phytochemicals with respect to the association with cancer/cancer prevention are still the carotenoids.

2.6.1 Dietary carotenoids

Carotenoids are a group of more than 600 compounds found in living organisms including plants, animals and bacteria which mostly consist of carotenes and xanthophylls. (Grosvenor & Smolin, 2002). Carotenes are a substance of hydrocarbon, whereas xanthophylls are oxygenated carotenoids containing alcohol, carbonyl, or other functional group (Murano, 2003). There are more than 40 carotenoids available (e.g. α -carotene, β -carotene, lycopene, lutein and xanthins) that can be metabolized by humans (Greenwald *et al.*, 2001). Carotenoids are known to provide antioxidant properties that have mostly been associated with a reduced risk for certain cancers, cardiovascular disease, macular degeneration and ageing. The most common form of carotenoids, the precursor of Vitamin A also known as β -carotene, a fat soluble vitamin (Francis, 1985).

Metabolism and bioavailability of carotenoids in the body depends on several factors that might influence the absorption of the substance. For instance, food processing methods and cooking could possibly cause mechanical breakdown of the plant tissue and thus affect the absorption into gastrointestinal mucosal cells. Not much is known about carotenoid mechanisms that are involved but the major site of carotenoid storage is in the adipose tissues (Rao & Rao, 2007). Among the factors influencing cancer growth, antioxidants such as tocopherol or β -carotene have been the most widely studied and seem to offer the best hope for growth inhibitions (Kritchevsky, 2003).

Studies have also shown that carotenoids are important in protecting against cancer due to their strong antioxidant capacity. This can be due to their ability of neutralizing free

radicals in the body. Moreover, the chain structure of β -carotene has the ability to promote normal cell differentiation and inhibition of cancerous aberrant cell growth and cell proliferation (Simon, 2002). Additionally, β -carotene has beneficial effects in terms of human immune function and enhancement of cell to cell communication (Greenwald *et al.*, 2001). Moreover, β -carotene was found to boost proliferation of T & B lymphocytes which act as a natural killer cell while helping to decrease chromosomal abnormalities. (Burri, 1997).

2.6.2 Sources

The main food sources of β -carotene are the yellow-orange coloured fruits and vegetables. Leafy greens such as broccoli and spinach as well as apricots, carrots, capsicum and tomatoes are some of the fruit and vegetable sources that are particularly rich in β -carotene.

2.6.3 Estimated dietary carotene intake

In order to understand whether a specific treatment from specific foods leads to an acceptable nutrient quality, further research is needed to study both human requirements and the amount of a particular nutrient present in food after normal preparation procedure. To measure the quantitative needs of human essential nutrients, Recommended Daily Allowances (RDA) is used as a guide by scientists in planning of diets, food nutrition labelling as well as for evaluating the nutritional adequacy of foods consumed (Tannenbaum *et. al.*, 1985). In Malaysia, the Recommended Nutrient Intake (RNI, 2005)

for men aged 16 years old and above as well as women aged 65 years and above are 600 μg of Vitamin A in their daily diet. On the other hand, the RNI for women aged 19 years up to 64 years is of 500ug Vitamin A, which can also be expressed as retinol equivalent (RE), where 1 RE = 1 μg retinol or 6 μg β -carotene (Tee *et. al.*, 1997).

2.6.4 Dietary carotene and cancer prevention

Most of the research studies on phytochemicals or vitamins with respect to the association with cancer or cancer prevention are β -carotene. Many researchers found strong associations of β -carotene and lower risk of lung cancer. Recent studies have also suggested protective effect of carotenoids against other cancer sites such as the oesophagus, stomach, colon, rectum, breast and cervix (Simon, 2002). Although the analysis of diet through foods is strongly recommended for public health purposes, the studies on macro and micro nutrients could offer comparable advantages in terms of understanding the mechanisms such as including the calculation of total energy intake with the disease of interest (Stefani *et. al.*, 1999b).

Studies on carotenoids have also been undertaken to determine if the colourful compounds were a cancer-protective agents. Clinical intervention trials involving human studies have reported the response rate of oral leukoplakia to β -carotene to be as high as 44-71% without significant toxicity. Similarly, a combination of 30mg of β -carotene, 1000mg ascorbic acid and 800IU of α -tocopherol per day for 9 months have also shown marked clinical improvement in 56% of patients (Khaodhiar & Blackburn, 2006). Supplementation with 30-60mg of β -carotene for approximately 6 months has also proven to be effective in

suppressing oral leukoplakia of up to 50-60% during the early phases of the disease (Naves & Moreno, 1998). Reviews of many epidemiological studies indicated that high intakes of β -carotene-rich vegetables and fruits or high blood concentrations of β -carotene found significant inverse association with the risk of lung cancer (Greenwald *et. al.*, 2001).

Thus far, there has not been much published data on the relationship between food intakes and oral cancer carried out in the Malaysian population. A preliminary study by Zain *et. al.*, (1997) which investigates the relationship of serum micronutrients level with oral cancer and pre cancer (OPC) prevalence among selected ethnic groups (Indian, Malays and Sarawakians) noted that serum level of alpha-tocopherol, zeaxanthin/lutein and β -carotene were found to be significantly lower in patients with oral cancer and pre cancer as compared to those without lesions (Zain, 2001). Further studies on specific micronutrients associated with the risk of oral cancer are very much needed to establish a proper dietary pattern since there is insufficient proof that these nutrients act as antioxidants.

Chapter 3

Materials and Methods

3.1 Study Design

This is a cross sectional study with case control design in a multi-centre hospital setting involving patients/subjects. The aim of this research is to determine the association between the mean intake of β -carotene as well as the dietary pattern with the risk of oral cancer in a Malaysian population. The Oral Cancer Research and Coordinating Centre (OCRCC) maintains a data bank on oral cancer cases and control subjects obtained from participating centres/hospitals. These were the Faculties of Dentistry Universiti Malaya (UM), Universiti Sains Malaysia (USM), Universiti Kebangsaan Malaysia (UKM) and the Ministry of Health, Malaysia, Specialist Clinic at General Hospital of Kuala Lumpur, Tuanku Ampuan Rahimah Klang (Selangor), Ipoh (Perak), Kota Bahru (Kelantan), Kuching (Sarawak) and Queen Elizabeth (Sabah).

All histologically diagnosed oral cancer cases in the participating centres/hospitals were considered eligible to be included in this study. Control patients were taken from healthy individuals attending these centres for minor ailments without the disease of interest. A brief overview of the methodology from collecting sample subjects to statistical analysis is presented in Figure 3.1.

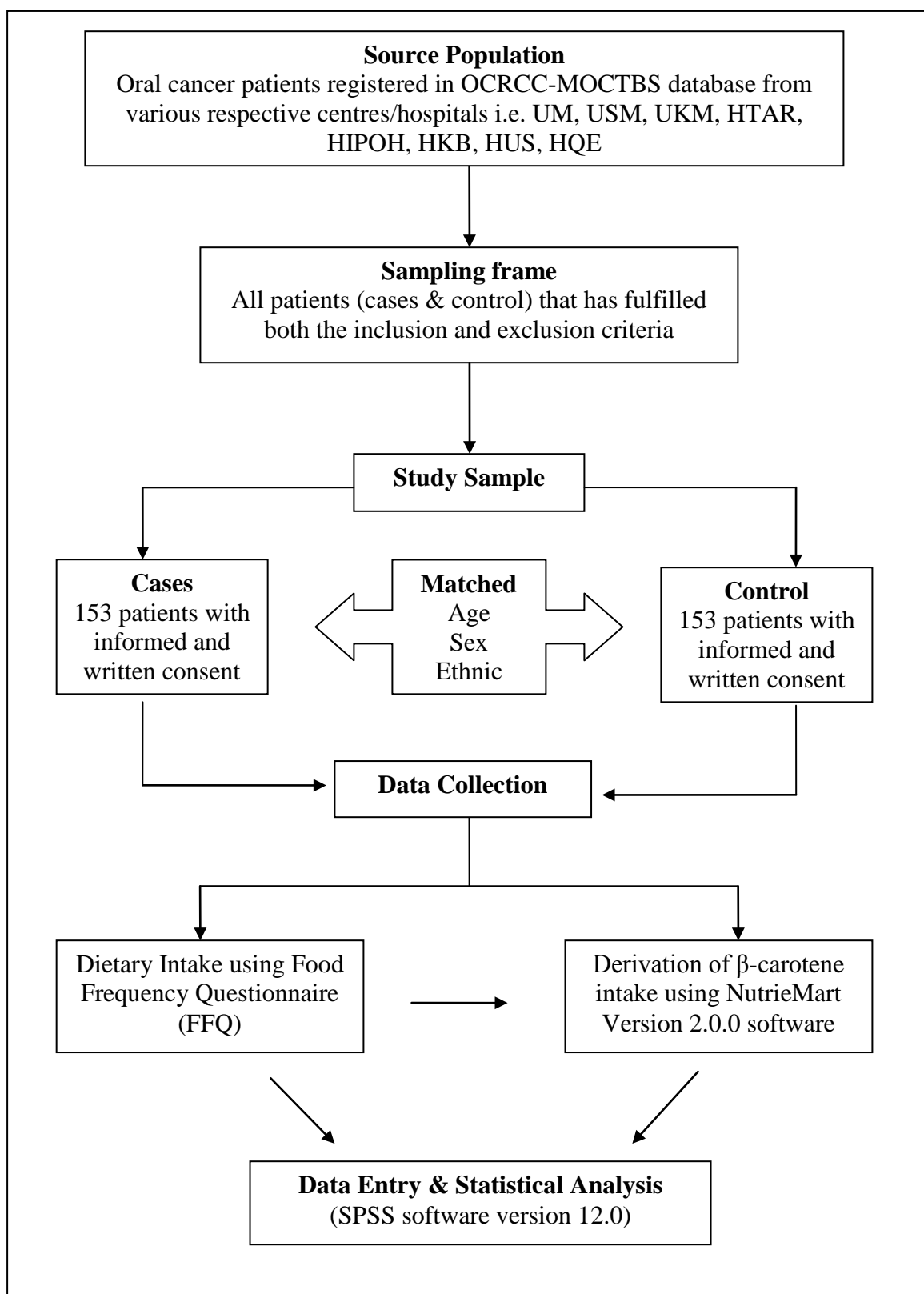


Figure 3.1 Flow chart illustrating research methodology for this study

3.2 Source population

The source population in terms of cases and control for this study was obtained from the database of OCRCC-MOCTBS (Malaysian Oral Cancer Database and Tissue Banking System). Each respondent's data contains information on socio-demographic background, risk habits which includes tobacco smoking, alcohol drinking, betel quid chewing as well as dietary intake, quality of life, diagnosis, clinical staging and histological grading which have been collected in a standardized manner. All information on respondents' daily food consumption intake was obtained by way of face to face interviews using trained personnel at each of the participating centres.

3.3 Sample

As diet may be influenced by multicultural differences, an equal sampling of cases and controls were taken from the 3 main ethnic groups i.e. Malays, Chinese and Indians that constitute the population of Malaysia. Gender and age (within five years) were also matched for each selected case and control in order to reduce bias in the study sample. The study sample was obtained from the source population that have fulfilled all inclusion and exclusion criteria as listed below.

3.3.1 Inclusion and Exclusion Criteria

i) Inclusion Criteria

Patients who were diagnosed pathologically with squamous cell carcinoma of the oral cavity (OSCC) at the nine selected centres were included in this study. For controls, all patients who do not have any cancer, potentially malignant lesions or other disease onset were eligible for inclusion. However, some control patients could be attending the centres for minor ailments. In this study, for both the cases and controls, only subjects with complete diet data set and information were included.

ii) Exclusion Criteria

Only Malaysian citizens were considered as subjects in this study. Subjects who had cancer or currently undergoing treatment were eliminated. This is to eliminate those with recurrent cancer. Subjects who had history of cardiovascular disease (CVD), hypertension, gastrointestinal tract disease (GI) and liver disease were excluded to reduce bias. Subjects with insufficient dietary information were also excluded.

3.3.2 Sample size

The sample size is based on past literature review from the nearest population of study in Jakarta, Indonesia where a prevalence of non exposure groups on dietary intake with oral cancer risk was 30% controls as well as considering an estimated relative risk or odds ratio (OR) of 2.0 (Amtha *et al.*, 2009). The sample size was then determined using Epi Info Version 3.5.1, with confidence interval (1- α) of 95%, power (1- β) of 80%, ratio of cases to control of 1:1 and significance level = 0.05.

Based on the above formula, the estimated sample size for this study would be 153 cases and 153 controls respectively. During the data collection period, 306 matched cases and controls (51 pairs of Malays, Chinese and Indians each) in the data bank were randomly selected to fulfil the required sample size. The sample size calculation is attached in Appendix A.

3.3.3 Subjects informed consent and ethical issue

An informed and signed consent was obtained from all the subjects who participated in this study. A copy of subject information sheet and signed informed consent for the research are shown in Appendix B.

This research protocol was approved by the Medical Ethics Committees of University Malaya, Kuala Lumpur [ethics code no. DF OP0306/0018/(L)] and endorsed by the Ministry of Health. This research was supported by the grant from the umbrella project

of Oral Cancer & Precancer in Malaysia – Risk Factors, Prognostic Markers, Genetic Expression & Impact Quality of Life, IRPA RMK 8 Project No: 06-02-03-0174 PR 0054/05-05. This current study formed part of the main project.

3.4 Variables

The dependent variable in this study is the oral cancer status. All risk factors associated with oral cancer are the independent variables and were categorized into social demographic factors, dietary food items/groups intake and β -carotene intake which are illustrated in Figure 3.2.

3.5 Measurement tools

This study was mainly based on secondary data obtained from the OCRCC-MOCTBS database where validity of information is crucial to ensure its reliability. Two different measurement tools were used to collect the required information as well as to quantify different exposure or the risk factors of interest.

The first measurement tool used for data collection was the semi-quantitative Food Frequency Questionnaire (FFQ) while the secondary data obtained from FFQ was the quantification of β -carotene intake computed using the NutrieMart Version 2.0.0 software. These two measurement tools were used to quantify the dietary intake of each respondent as it is more appropriate to estimate food types and frequencies in making relative between

group comparisons and also for categorising subjects into bands of consumption (Moynihan *et. al.*, 2009).

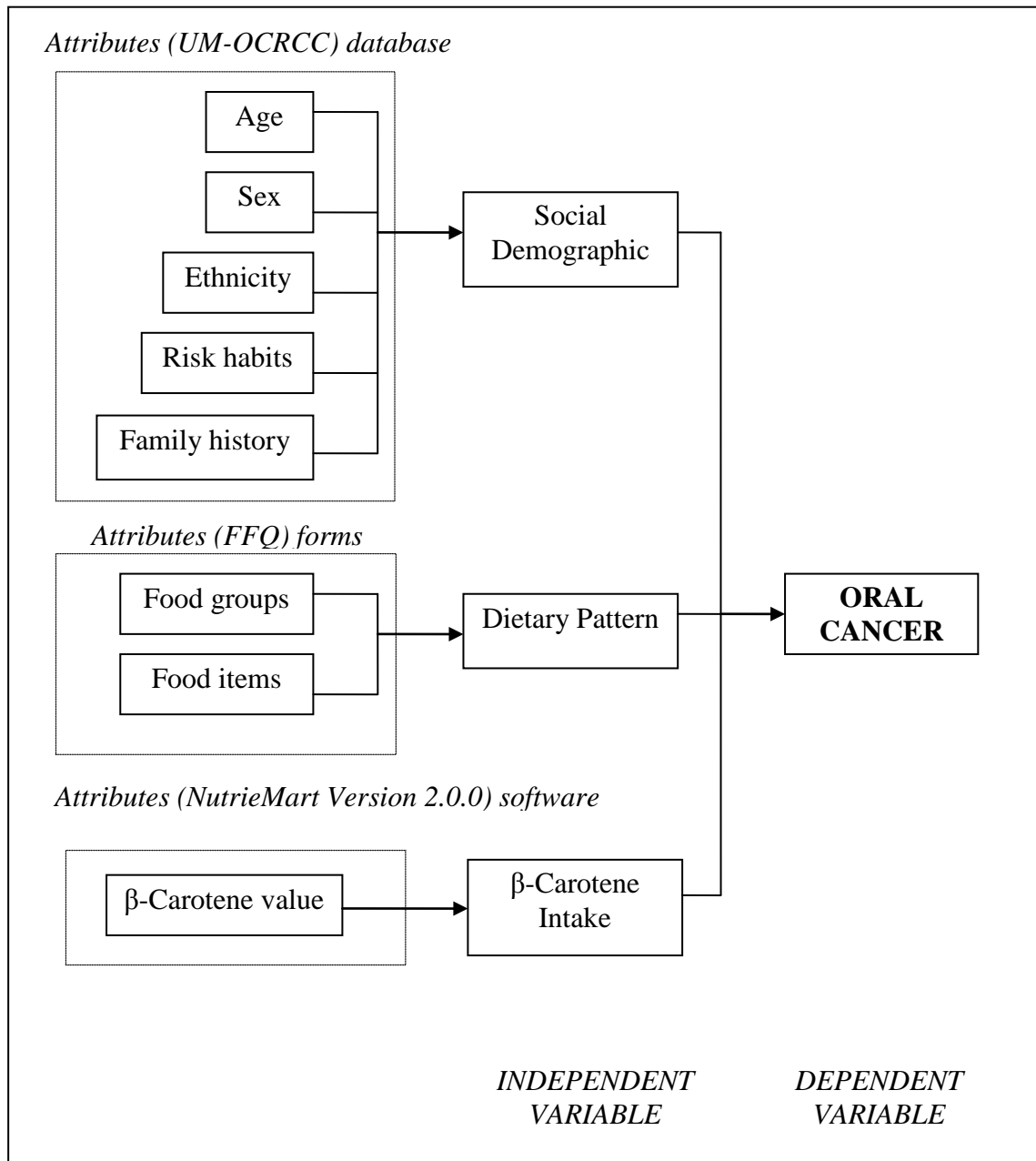


Figure 3.2: Independent and dependent variables in the study framework

3.5.1 Food Frequency Questionnaire (FFQ)

A structured food frequency questionnaire (FFQ) which have been developed and validated for the Malaysian population to collect information on the subjects' dietary intake was used to access patterns of food intake of each respondent. The FFQ form consisted of ninety-nine food items and beverages, which were grouped into 9 different sections namely: (1) dairy food; (2) fruits; (3) vegetables; (4) eggs, meat, fish, shellfish and products; (5) bread, cereals and starches; (6) beverages; (7) sweets, baked goods and traditional Malaysian *kuih*; (8) fermented or salted foods; and (9) processed foods.

The FFQ form also specify commonly used unit or serving sizes described by using natural portions or standard weight and volume measures in the study population. The complete set of the FFQ dietary intake form that include portions and average consumption of all listed food items mentioned is presented in Appendix C.

This is an interviewer guided questionnaire where trained personnel from each of the participating centres asked the subjects to indicate the average frequency of food intake for each of the dietary item as shown in Table 3.1. For oral cancer patients, they were required to recall their usual estimated dietary intake 1 year prior to diagnosis whereas in control respondents, they were asked to recall their usual estimated dietary intake from the previous year.

Table 3.1: Classification of food items according to the FFQ forms

Food groups	Food items
Dairy	Skim/low fat milk, whole milk, sweetened/condensed milk, ice-cream, yoghurt, cheese, margarine, butter, ghee
Fruits	Papaya, banana, mango, watermelon, apples, apple juice, orange, orange juice, guava, other fruit juices, pineapple, jackfruit, rambutan, durian, 'langsar'
Vegetables	Tomatoes, tomato sauce, red chilli sauce, soy beans, string beans, cabbage, cauliflower, raw carrot, cooked carrot, mix vegetables, winter melon, brinjal, yams, raw spinach, cooked spinach, mustard, 'ulam', celery, bamboo shoot
Eggs, Fish, Meat, Seafood (Meat/by-products)	Eggs, chicken, beef, internal, pork, mutton, dark meat fish (mackerel/sardine), cuttlefish/prawns, crab/cockles
Bread, Cereals and Starches (Starches)	White bread, wholemeal bread, brown rice, white rice, coconut milk rice, 'dagang' rice, corn, noodles, 'idli', 'capati' bread, 'canai' bread, 'dosai'
Beverages	Canned/carbonated drinks, coffee, tea
Sweets, Baked goods and Traditional Malaysia <i>kuih</i> (Snacks)	Chocolate (bars/snickers), muffins/biscuits, cream cracker biscuits, sultana/Marie biscuits, 'pulut panggang', curry puffs, 'cokodok pisang', 'murtabak', 'keropok lekor', jams/jellies, nuts, pepper, salt
Fermented or Salted	Fish sauce, fermented shrimp, shrimp paste, salted fish, Chinese salted fish, salted duck egg, fermented durian, pickled chilli, pickled fruits, soy sauce
Processed	Bacon, processed meat (sausages/salami/hotdog), burger, pizza, sandwich, French fries, instant noodles, nuggets (fish/chicken), instant spices

For each individual food items, subjects were to indicate their intakes of each food items which were categorized by nine consumption group termed as (1) “never or less than once per month”, (2) “1-3 per month”, (3) “1 per week”, (4) “2-4 per week”, (5) “5-6per week”, (6) “1 per day”, (7) “2-3 per day”, (8) “4-5per day” and (9) “6+ per day”. For each of the food items taken by the individual subjects, a score will be tabulated based on their daily equivalent to determine the estimated food intake.

3.5.2 NutrieMart Version 2.0.0

Nutrient composition database is used when dietary data are converted to nutrient intake data. Such database includes the description of food, food code and the nutrient composition. The NutrieMart Version 2.0.0 developed by Custommedia, (2007) is a reliable and validated computer software programmed for the Malaysian Population which was used to supplement quantification value of the daily nutrient intake from an individual food consumption. This nutritional software is also specially designed to include both nutrient composition database and convert individual response to specific nutrients by quantifying a substantial amount of macro and micro nutrients such as carbohydrate, fat, protein, vitamin, mineral and others from the total intake of foods consumed.

3.6 Data collection

Data was obtained from the database of MOCTBS in OCRCC. A summary of data collection procedure for the patient information is presented below in Figure 3.3

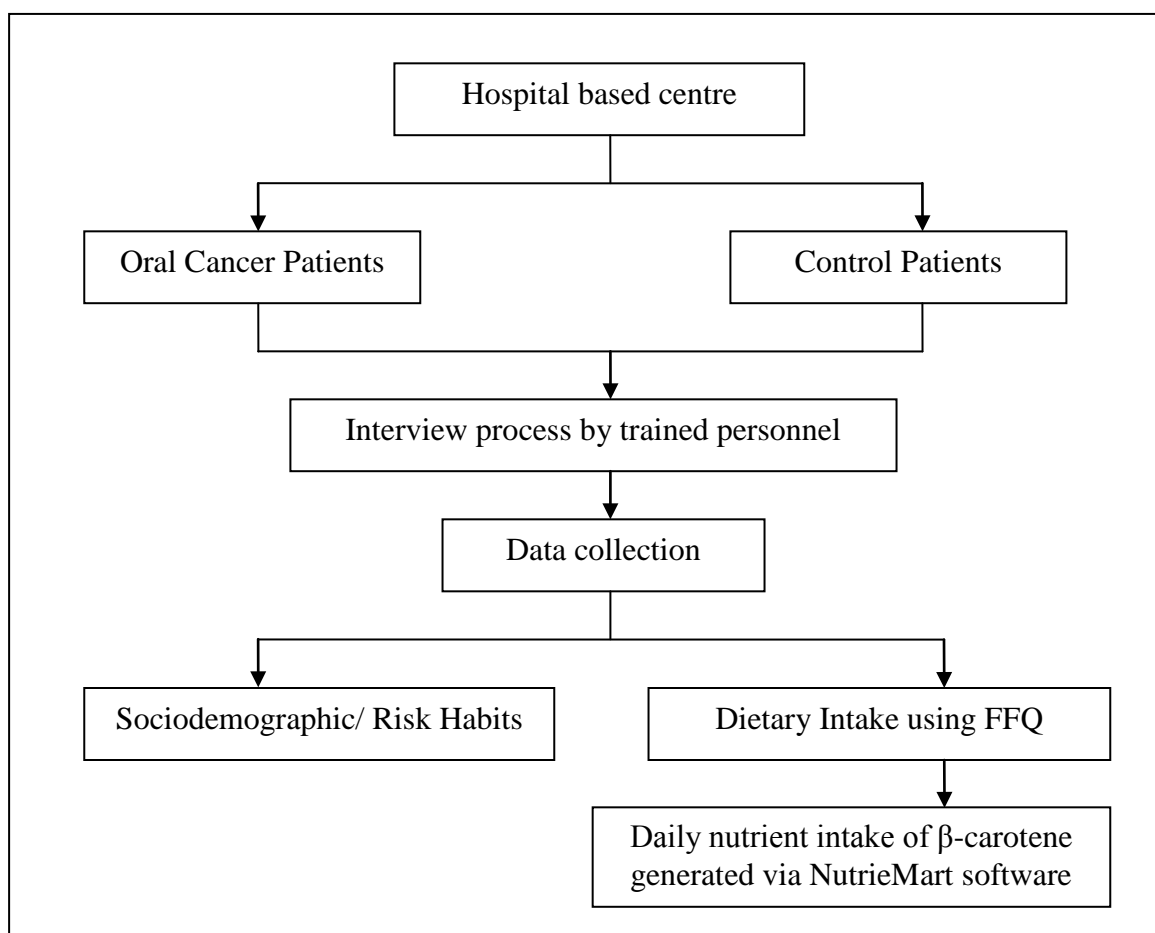


Figure 3.3 Data collection process from respondents

Socio-demographic information of case and control patients such as habits for tobacco smoking, alcohol drinking, betel quid chewing and family history of cancer were obtained from the database. The sources of dietary data were obtained from the FFQ forms and the intake of carotene was computed and generated via NutrieMart Version 2.0.0 software. For further clarification and understanding of the whole data collection process that involves various sources of data or information, a summarized version of the process is presented in Table 3.2.

Table 3.2 Sources obtained from the data collection process

Variable	Sources		
	OCRCC database	FFQ forms	NutrieMart Software
Dependent variable			
Oral cancer patients	x		
Control patients	x		
Independent variable			
Sociodemographic	x		
Dietary pattern		x	
β-Carotene intake			x

3.6.1 Information on dietary intake (FFQ)

All information pertaining to the dietary intake was extracted in two stages. The first stage includes utilization of secondary data from the FFQ which was subjected to factor analysis where all food groups were analysed to determine the inter-relationship among each of the food items. These data obtained from FFQ was numerically transformed based on the mean daily equivalent score and were computed for quantitative meaningful data (Northstone *et. al.*, 2008). For each of the food items, the frequency of food consumption is converted into daily equivalent score as presented in Table 3.3.

Table 3.3: Frequency of food consumption and daily equivalent scores

Frequency of food consumption	Daily equivalent score
never or less than once per month	0/7 day = 0
1-3 per month	1/30 day = 0.03
1 per week	1/7 day = 0.14
2-4 per week	2/7 day = 0.28
5-6 per week	5/7 day = 0.71
1 per day	1
2-3 per day	2
4-6 per day	4
6+ per day	6

3.6.2 Information on dietary β -carotene intake (NutrieMart Version 2.0.0)

For the purpose of this study, the daily equivalent scores which were based on the FFQ for the estimated food intake were then entered into the NutrieMart software to compute the daily nutrient intake for each of the food items. All nutrient and food composition information from the NutrieMart software was referred from the Nutrients Composition of Malaysian Foods guidebook compiled by Tee *et. al.*, (1997). This guidebook consists of basic macro and micronutrients as well as minerals grouped into different sections and it include almost all types of Malaysian food. The final output from the NutrieMart software as shown in Appendix D would be the total energy and daily nutrient intake for each of the food items that include dietary β -carotene as well. This auto-generated values of β -carotene for each respondent from the software were extracted and keyed into the data worksheet for further statistical analysis.

3.7 Statistical Analysis

The Statistical Package for Social Sciences (SPSS) Version 12.0 software was utilized for all statistical analysis. Analysis was done based on the specific objectives of the research study. Data were cleaned and checked before proceeding to examine the frequencies and distribution of the dataset. Descriptive statistics were used to describe all dependent and independent variables. All continuous data were tabulated using mean and standard deviation whereas all categorical data were calculated as frequency and percentage.

In order to achieve the objectives of this study, several statistical methodologies were employed where each of the food items listed in the 9 main food groups will undergo a series of preliminary test to determine the reliability of the scale matrixes (food items) before performing the required analysis. After which, factor analysis was applied to study and describe the dietary pattern from the food items/groups respectively. In order to determine the consumption of food items/groups as well as the mean intake of β -carotene that could be associated with oral cancer risk, logistic regression analysis was used.

3.7.1 Reliability test

The reliability of a scale measurement indicates how free the study is from random error. In order to minimize the potential error, reliability test was conducted to see the internal consistency within the food items and the degree in which these items would make up the scales measuring the same underlying attributes of the given food groups. The 99

food items from the FFQ form were then regrouped to check the internal reliability on each of the food items listed.

The most common indicators used for this test would be the Cronbach's alpha coefficient and a value of above 0.7 would be acceptable. This statistics provides an indication of an average correlation among all the items that would make up the scale where the values range from 0-1, with the highest value indicating greater reliability (Pallant, 2005). However, a low Cronbach's alpha coefficient value means that it has less reliability due to the sensitivity of the items that are loaded in that scale. Scales loaded with fewer than ten items tends to have lower Cronbach's alpha coefficient value (Pallant, 2005). Peat (2001) had also noted that Cronbach's alpha values between the range of 0.4-0.6 were considered as moderately reliable.

3.7.2 Factor Analysis on Dietary Pattern

The Principal Component Analysis (PCA), transforms a large data set of correlated variables (food items/groups) into smaller set of non-correlated variables (Marchioni *et. al.*, 2005). This was followed by a multivariate statistical modelling using Factor Analysis (FA). Factor analysis was applied to identify the combination of food items consumed by respondents in this study group. It was noted that such factors would correspond to the indicators, and considered simultaneously related to one another (Marchioni *et. al.*, 2005 and Marchioni *et. al.*, 2007). The main goal of this technique is to identify the underlying structure in a data matrix, by summarizing and reducing the data to supply a synthetic measurement of the diet (Marchioni *et. al.*, 2005).

A series of tests to determine the measurement of sample adequacy was verified using Kaiser-Meyer-Olkin (KMO) and Bartlett Test of Sphericity (BTS) was used to test the presence of correlation between variables. When the requirements were met with KMO >0.600 and BTS <0.05, the sample was considered adequate for FA (Pallant, 2005). This was then followed by exploratory FA being applied to the food groups to find the principal factors that account for the maximum fraction in the dataset.

Factors to be retained were based on a graphical scree plot determined by Kaiser Criterion (if the eigenvalue is more than 1). Factor loadings were derived from the orthogonal rotation using varimax method where it categorized food intake into positive and negative loadings that provide information indicating the type of food pattern or nutrient which was directly or inversely correlated with the risk factor (Stefani *et. al.*, 2008). FA was also used to analyze the measurements of correlations between factors to enable easier interpretation for each of the independent factor (Marchioni *et. al.*, 2007 and Amtha *et. al.*, 2009).

Identifying significant factor loadings or correlation matrix for each variable was done using BMDP statistical software (1993). Factor loadings of 0.35 (for sample size of at least 250-350) would be considered as significantly contributing to the factors. Labels were given to reflect each factor/food groups which was highly represented. However when a food group was loaded on more than one factor, only the factor with the highest loadings was considered for factor naming (Marchioni *et. al.*, 2007). Hence, the dietary patterns which were explored from FA were then categorized into tertiles based on the study population and the risk estimates only at the highest tertiles. After which, the dietary

patterns will be subjected to multiple conditional logistic regression univariate model to obtain the odds ratio (OR) where confounding variables were adjusted for habits such as tobacco smoking, alcohol consumption, and betel quid chewing.

3.7.3 Logistic Regression

Logistic regression analysis is a widely used technique to yield odds ratio (OR) by measuring the association between a risk factor of interest and the disease outcome in epidemiology studies. An alpha level of 0.05 was used as indicator for statistical significance and Goodness of Fit model was calculated based on Hosmer and Lemeshow Test where a poor test fit is indicated by a significance level of less than 0.05.

3.7.3.1 Conditional Logistic Regressions for Dietary Pattern

To determine the association between dietary pattern derived from FA and oral cancer risk, conditional logistic regression models were performed. For multivariate analysis, control variables with p-value less than or equal to 0.05 in univariate analysis were retained. Variables that remained significant after adjustment of confounding factors were kept in the model presented as OR estimate by method of maximum likelihood. The variables were then introduced into the regression model using stepwise and enter procedure. The corresponding 95% confidence interval (CI) was based on the standard error of coefficient estimated and p-value for trend was also obtained based on each factor/food group which was entered as continuous variables into the model (Stefani *et. al.*, 2008).

3.7.3.2 Conditional Logistic Regressions for β -Carotene Intake

In this study, the dietary value of β -carotene intake as derived from NutrieMart software was divided into two different categories namely high intake of β -carotene or low intake of β -carotene which was based on the Recommended Nutrient Intakes (RNI), 2005 for Malaysia. The National Coordinating Committee on Food and Nutrition (NCCFN), a technical working group on nutritional guideline noted that the RNI for men aged 16 years and above and women aged 65 years and above is 600 μ g of Vitamin A in their daily diet. However, the RNI for women aged 19 to 64 years is 500ug Vitamin A.

Logistic regression analysis was then used to identify significant association which relates to high intake of β -carotene (above RNI) or low intake of carotene (below RNI) on oral cancer risk. Meanwhile, conditional logistic regression is used to control for potential confounding factors. 95% CI of the OR were used to estimate precision of the coefficient to its standard error in order to make inferences to the study population (Zheng *et. al.*, 1993).

Chapter 4

Results

This chapter presents the results of the analyzed data set obtained from the OCRCC-MOCTBS as well as the data computation of the β -carotene intake from case and control subjects.

4.1 Subjects recruited for the study

A total of 306 cases and controls were randomly selected from the database during the study period, fulfilling the required sample size. All case and control subjects were obtained based on the inclusion and exclusion criteria set, after which it was then matched for gender, age (within 5 years) and ethnicity.

4.1.1 Socio-demographic characteristics of the sample

The socio-demographic characteristics and the risk habits of the study sample are shown in Table 4.1. The result of the statistical analysis is attached in Appendix E.

The age range for oral cancer patients (case) in this study was 18-82 years (mean age of 52.4 ± 12.8) while that of the control patients was 17-81 years (mean age of 52.4 ± 12.6). The prevalence of oral cancer in the sample increases with increasing age group. More than 60% of the cancer cases were seen in ≥ 50 years age group and males accounted for slightly more than half of the study sample.

Significance differences were detected in all three risk habits that includes tobacco smoking, alcohol drinking and betel quid chewing in the sample. The proportion of tobacco smokers (43.1%), alcohol drinkers (31.4%) and betel quid chewers (32.0%) among the cases were significantly higher as compared to the controls of 17.6%, 13.7% and 17.6% respectively.

Although the prevalence of oral cancer was higher in those who had a past family history of cancer as compared to those who do not with a ratio of 6:4, the difference was not statistically significant.

Table 4.1: Socio-demographic characteristics and risk habits of subjects

Parameter	Control N=153 (%)	Cases N=153 (%)	χ^2 statistics (df)	p-value
Age (years)				
< 35 years old	14 (9.2)	16 (10.5)	0.151	0.927
35-49 years old	42 (27.5)	41 (26.8)		
≥ 50 years old	97 (63.4)	96 (62.7)		
Gender				
Male	81 (52.9)	81 (52.9)	0.000	1.000
Female	72 (47.1)	72 (47.1)		
Ethnicity				
Malay	51 (33.3)	51 (33.3)	0.000	1.000
Chinese	51 (33.3)	51 (33.3)		
Indian	51 (33.3)	51 (33.3)		
Risk Habits				
a) Smoking status				
No	126 (82.4)	87 (56.9)	23.496	0.000
Yes	27 (17.6)	66 (43.1)		
b) Alcohol drinking status				
No	132 (86.3)	105 (68.6)	13.641	0.000
Yes	21 (13.7)	48 (31.4)		
c) Betel-quid chewing status				
No	126 (82.4)	104 (68.0)	8.473	0.004
Yes	27 (17.6)	49 (32.0)		
Family History of Cancer				
No	129 (84.3)	118 (77.1)	2.541	0.111
Yes	24 (15.7)	35 (22.9)		

4.1.2 Association between risk habits and risk of oral cancer

Table 4.2 summarized the results of simple logistic regression (SLR) analysis for the association between risk habits and oral cancer risk at univariate level. All statistical analysis generated for socio-demographic parameters in this sample is attached in Appendix F.

Significant associations were found between tobacco smoking, alcohol drinking as well as betel quid chewing and the risk of oral cancer. Habitual smokers had significantly 3.5 times higher risk of having oral cancer than non smokers. Similarly, a significant increased risk of about 3 times was also detected among subjects who consumed alcohol and about two times among those who chewed betel quid as compared to those who did not.

No association was found between family history of cancer with oral cancer risk. Although those with family history of cancer showed 1.6 times higher risk of developing oral cancer as compared to those without the family history, the findings was not statistically significant.

Table 4.2 Association between risk habits and oral cancer risk

Parameter	Crude OR	95% CI	χ^2 statistics (df)	p-value
Risk Habits				
a) Smoking status				
No	1.000			
Yes	3.540	2.095-5.982	22.315	0.000
b) Alcohol drinking status				
No	1.000			
Yes	2.873	1.620-5.098	13.023	0.000
c) Betel-quid chewing status				
No	1.000			
Yes	2.199	1.285-3.761	8.277	0.004
Family History of Cancer				
No	1.000			
Yes	1.594	0.896-2.837	2.516	0.113

4.2 Reliability analysis of food groups

The variables from the FFQ form which consisted of 99 food items under nominal scale in each construct was then subjected to reliability testing to facilitate further analysis. The Cronbach alpha coefficient was computed to check internal reliability and to ensure that the employed scales (food items) measured consistently what it was intended to measure for the 9 food groups. The value of mean, standard deviation and reliability test from the analyzed food groups is illustrated in Table 4.3. The Cronbach alpha showed low values within two food groups, namely “dairy foods” (0.348) and “starches” (0.357) with “beverages” (0.219) having the lowest value.

Table 4.3: Cronbach alpha values for 9 different food groups

Food groups	No of Items	N	Mean	SD	Cronbach Alpha
Dairy	9	306	16.92	5.804	0.348
Fruits	15	306	27.56	9.116	0.855
Vegetables	19	306	43.12	11.524	0.815
Meat/by products	9	306	19.59	5.803	0.656
Starches	12	306	29.28	6.540	0.357
Beverages	3	306	9.05	3.900	0.219
Snacks	13	306	25.11	9.273	0.790
Fermented or Salted	10	306	14.58	4.997	0.708
Processed	9	306	13.94	4.965	0.718

4.3 Factor analysis of dietary pattern

The observed Kaiser-Meyer-Olkin (KMO) which is a measure of sampling adequacy was 0.755 (exceeding the recommended value of > 0.600) and the Barlett's test of sphericity was statistically significant ($0.000, < 0.05$), thus, supporting the adequacy of study sample for factor analysis. Principal Component Analysis (PCA) identified the presence of three major components based on the Kaiser Criterion (where *Eigenvalues* was more than 1). However, an inspection of the scree plot (Figure 4.1) revealed a little break from the third and fourth component. Since factor analysis was used as a data exploration technique, it was decided to retain four components instead of three for further investigation and exploration. These four components accounted for 69.4% of the variability within the sample.

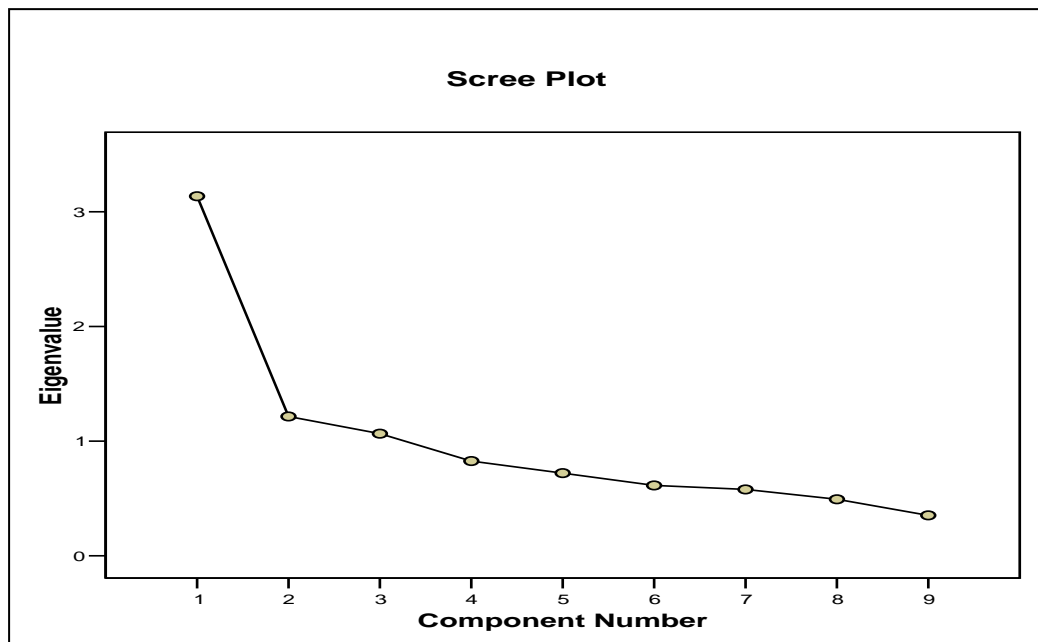


Figure 4.1: Scree plot showing *Eigenvalues* for 9 major food group/components, extracted from Factor Analysis of data obtained from the food frequency questionnaire

Table 4.4 shows the loading factor obtained after orthogonal varimax rotation which resulted in better interpretation and reporting purpose. Factor loadings obtained for each dietary variable in each factor of more than 0.40 have been highlighted and considered as having significantly contributed to the factor. In each food groups where cross loading arose, only the highest loading from each component was considered as it represented the underlying nature of that component.

Table 4.4: Food loadings of four dietary patterns identified in the study population

Food groups	Dietary pattern				Communality
	Modern	Prudent	Combination	Traditional	
Processed	0.889	0.049	0.063	0.088	0.804
Snacks	0.727	0.316	0.181	0.133	0.679
Fruits	0.281	0.694	0.233	-0.234	0.669
Vegetables	0.025	0.754	0.294	0.247	0.717
Dairy	0.397	0.553	-0.403	0.299	0.715
Meat/by-product	0.407	0.112	0.657	0.171	0.639
Fermented or Salted	0.019	0.225	0.788	0.060	0.676
Starches	0.316	0.279	0.159	0.597	0.559
Beverages	0.031	-0.056	0.051	0.882	0.784
Eigenvalues	3.135	1.215	1.065	0.826	
% explained variance	34.84	13.50	11.84	9.18	
% cumulative variance	34.84	48.34	60.17	69.35	

The first component (factor), which accounted for 34.8% of the total variance, is labeled as ‘modern’. Processed foods and snacks fall into this component. The second component explained 13.5% of the total variance. This factor was loaded with intake of vegetables, fruits, and was termed as ‘prudent’. The third component accounted for approximately 11.8% of the total variance. This component was loaded with intakes of dairy, meat and by product, as well as fermented or salted foods and was labeled as ‘combination’. The fourth factor explained about 9.2% of the total variance and was loaded with intake of beverages and starches. This fourth component (factor) is termed as ‘traditional’.

The highest communality was shown in ‘processed foods’ (0.804) which indicates that this variable has much in common with other variables taken as a group and loaded in the first factor which was labeled as ‘modern’.

4.3.1 Reliability analysis within the four dietary components

In order to ensure that these 4 food components/factors ‘fall together’ after undertaking factor analysis, reliability test was again conducted to check internal consistency. This would indicate much stronger evidence in support of collapsing different food items that fall within the same food group or component. Table 4.5 shows the observed results and the values that were obtained for the four different dietary components which showed moderate consistency.

Table 4.5: Cronbach alpha values for the four identified dietary patterns

Dietary components	No of Items	N	Mean	SD	Cronbach Alpha
Modern	22	306	39.05	13.219	0.855
Prudent	34	306	70.68	18.500	0.889
Combination	28	306	51.09	11.777	0.690
Traditional	15	306	38.32	8.585	0.464

4.4 Dietary pattern and risk of oral cancer

The Chi Square test, (χ^2) for the upper limit of intake in the four food components (modern, prudent, combination and traditional) that were retained from factor analysis was performed and it was observed that only one dietary pattern namely “combination ($p=0.034$, $p<0.05$)” was statistically significant whereas for “traditional ($p=0.072$, $p<0.05$)” pattern, a marginally significant risk was noted which could be contributing to the development of oral cancer as shown in Appendix G.

Table 4.6 illustrates the upper limit intake for each type of the food groups retained from factor analysis as well as the corresponding univariate analysis and adjusted odds ratio after allowing for confounding factors which were the risk habits of smoking, alcohol consumption and betel quid chewing (Appendix H).

Table 4.6: Univariate Analysis: Odds ratio and 95% CI for each type of food groups defined by Factor Analysis

Factor	Score	Crude OR (95% CI)	Adjusted OR (95% CI)	*p-value
Modern	1 st (≤ 1.00)	1.000	1.000	0.884
	2 nd (1.01-2.72)	0.760 (0.438-1.317)	0.805 (0.444-1.458)	
	3 rd (2.73+)	0.873 (0.504-1.510)	0.884 (0.490-1.595)	
Prudent	1 st (≤ 2.21)	1.000	1.000	0.212
	2 nd (2.22-4.08)	0.575 (0.331-1.002)	0.641 (0.353-1.163)	
	3 rd (4.09+)	0.674 (0.388-1.171)	0.685 (0.378-1.240)	
Combination	1 st (≤ 2.12)	1.000	1.000	0.006
	2 nd (2.13-3.80)	1.150 (0.663-1.996)	1.378 (0.755-2.516)	
	3 rd (3.81+)	2.010 (1.149-3.516) ^a	2.328 (1.271-4.263)	
Traditional	1 st (≤ 3.35)	1.000	1.000	0.054
	2 nd (3.36-4.88)	1.219 (0.702-2.116)	0.956 (0.524-1.746)	
	3 rd (4.89+)	1.883 (1.080-3.283) ^a	1.804 (0.991-3.286)	

^a (p<0.05)

*adjusted for risk habits (smoking, alcohol consumption and betel quid chewing)

Analysis at the univariate level showed that consumption of both ‘combination’ (aOR=2.328, 95% CI=1.271-4.263, $p<0.05$) and ‘traditional’ patterns (aOR=1.804, 95% CI=0.991-3.286, $p<0.05$) in the highest tertile were associated with significant increased risk of about two-fold after allowing for confounding factors.

Consumption in the highest tertile of ‘prudent’ pattern (aOR=0.685, 95% CI=0.378-1.240, $p<0.05$) displayed protective effects of about (32%) in relation to oral cancer risk. However, it was found to be insignificant after adjusting for risk habits. Similarly, food consumption of the highest tertile for ‘modern’ pattern (aOR=0.884, 95% CI=0.490-1.595, $p>0.05$), showed a non-significant reduced risk as well after allowing for confounding factors.

Since a variety of food were consumed by the general population, it would be interesting to further investigate the interaction among these food groups. Subsequently, Table 4.7 illustrates the upper limit intake for four different types of food groups that were retained from factor analysis as well as the corresponding multivariate analysis and the adjusted odds ratio (Appendix I) that were obtained after allowing for confounding factors which were the risk habits of smoking, alcohol consumption and betel quid chewing.

Table 4.7: Multivariate Analysis: Odds ratio and 95% CI for different food groups defined by Factor Analysis

Factor	Score	Crude OR (95% CI)	Adjusted OR (95% CI)	*p-value
Modern	1 st (≤ 1.00)	1.000	1.000	0.466
	2 nd (1.01-2.72)	0.674 (0.345-1.316)	0.706 (0.343-1.456)	
	3 rd (2.73+)	0.818 (0.454-1.473)	0.790 (0.418-1.490)	
Prudent	1 st (≤ 2.21)	1.000	1.000	0.102
	2 nd (2.22-4.08)	0.477 (0.247-0.919)	0.513 (0.254-1.038)	
	3 rd (4.09+)	0.527 (0.284-0.976) ^a	0.574 (0.295-1.117)	
Combination	1 st (≤ 2.12)	1.000	1.000	0.001
	2 nd (2.13-3.80)	1.610 (0.829-3.125)	2.116 (1.012-4.422)	
	3 rd (3.18+)	2.428 (1.325-4.450) ^a	2.986 (1.551-5.746)	
Traditional	1 st (≤ 3.35)	1.000	1.000	0.027
	2 nd (3.36-4.88)	1.489 (0.777-2.856)	0.999 (0.491-2.029)	
	3 rd (4.89+)	2.318 (1.265-4.245) ^a	2.078 (1.088-3.970)	

^a(p<0.05)

*adjusted for risk habits (smoking, alcohol consumption, betel quid chewing)

The results of multivariate analysis reveal that consumption of both ‘combination’ (aOR= 2.986, 95% CI=1.551-5.746, $p<0.05$) and ‘traditional’ patterns (aOR=2.078, 95% CI=1.088-3.970, $p<0.05$) in the highest tertile posed significant increased risk of about 3-fold for ‘combination’ pattern as compared to about 2-fold for ‘traditional’ pattern. The result was obtained after allowing for the same confounding factors mentioned.

Consumption in the highest tertile of the ‘prudent’ pattern (OR=0.527, 95% CI=0.284-0.976, $p<0.05$) displayed protective effects of about (47%) in relation to oral cancer risk before adjusting for the variables mentioned. However, the protective risk of developing oral cancer was slightly weak for ‘prudent’ pattern (aOR=0.574, 95% CI= 0.295-1.117, $p>0.05$) as it shows a non-significant reduced risk after adjusting for the confounding factors.

Multivariate analysis of dietary consumption in the highest tertile for ‘modern’ pattern (aOR=0.790, 95% CI=0.418-1.490, $p>0.05$) showed a non-significant reduced risk after allowing for the confounding factors mentioned, portraying similar results as observed in the univariate analysis.

4.5 Dietary β -carotene intake and risk of oral cancer

Table 4.8 summarizes the effect of dietary β -carotene intake and oral cancer risk at univariate level which was analyzed using simple logistic regression (SLR). The result obtained from Chi Square analysis shows that there was no significant association (0.599, $p>0.05$) between dietary carotene intake with oral cancer risk (Appendix J).

High intake of carotene was found to be associated with lower risk of having oral cancer by 17% (OR=0.83, 95% CI=0.417-1.656, $p>0.05$) although this was statistically not significant. According to (RNI, 2005), the intake of carotene was low for both the cases (86.9%) and controls (88.9%) respectively. A higher percentage of control subjects (13.1) were observed to have consumed β -carotene daily as compared to cases (11.1).

Table 4.8 Association between dietary β -carotene intake and oral cancer risk

Dietary β-Carotene Intake	Case (%) (N=153)	Control (%) (N=153)	Crude OR (95% CI)	χ^2 statistics (df)	p-value
Low (below RNI)	136 (88.9)	133 (86.9)	1.000	0.277	0.599
High (above RNI)	17 (11.1)	20 (13.1)	0.831 (0.417-1.656)		

Chapter 5

Discussion

5.1 Limitation of this study

This study aimed to determine the association between dietary pattern as well as β -carotene intake with the risk of oral cancer. It is a hospital based case-control study where subjects were recruited from participating centres/hospitals. A total of 153 matched cases and controls were randomly selected from the OCRCC-MOCDTBS. Data was collected using a structured questionnaire and then statistically analyzed for inferences. Some limitations were encountered during the course of this study.

5.1.1 Sample selection

This study was based on a case-control matched for age, gender and ethnicity which have duly fulfilled the required equal sample size. Matching is undoubtedly the most useful techniques for small studies as sample selection bias may easily arise if and unless special precautions are undertaken to assure uniformity of the confounding variables (Grobbee & Hoes, 2009). In this study, age, gender and ethnicity were chosen as the confounding factors which had to be matched, because it was assumed that these factors were related to the study exposure not to the disease. In Malaysia, multiple ethnicities influence the food choices and preparation of dishes. Each ethnic group comprising of the Malays, Chinese and Indians practises different dietary habits according to their respective cultures and religions. Thus, matching this variable would minimize the confounding bias in this study.

As mentioned earlier, this study utilized a sampling method in which one control was matched for each case.

The selection of comparable controls subject is very important as it is easily subjected to estimation bias if the controls selected do not reflect the distribution exposure of the population. However, most studies in this field usually depend on the use of hospital-based controls due to the difficulties in getting sample subjects from community controls and limited duration of the study (Toporcov *et. al.*, 2004 and Petridou *et. al.*, 2002). In this study, hospital-based controls were recruited for comparison purposes. To overcome the selection biases associated with hospital-based controls, suitable study design such as matched case-control and statistical analysis such as conditional logistic regression were applied to minimize the error of the results obtained.

5.1.2 Recall biases

This study was based on data collection via patients' recall as they were required to recall past food history. Therefore, measurement error may occur especially in data reporting as it usually depends on the patient's memory and their willingness to provide full co-operation with the interviewers (Carpenter, 2006). For example, control subjects maybe more likely to under report their consumption whereas case subjects tend to pose more attention to their food intake. Other factors may play a role in terms of recalling past dietary intake such as memory loss, current food intake, education level and social background.

All of the above mentioned factors could possibly influence the validity of the results and may underestimate or overestimate the level of agreement with the actual usual intake (Thompson & Subar, 2008). In order to minimize bias through the interview process, all personnel attached to the centres/hospitals had undergone training on the collection of the required data. The questionnaire format was made simple for interviewer or translator to facilitate effective communication response from the sample subjects, some of whom were illiterate.

5.1.3 Limited resources on diet and nutrition information

Not many studies have been conducted on dietary pattern and its association with oral cancer as summarized in Table 5.1. Availability of the dietary pattern information is crucial to serve as a reference point for this study. However, due to the limited references in Asian countries particularly, therefore, this study relies heavily on the Western dietary pattern which in fact does not reflect the real scenario of Asian countries especially in Malaysia.

Table 5.1 Summary of studies on dietary pattern

Study Location	Study Design	Outcome of dietary pattern	Cancer Site	References
Sao Paulo, Brazil	Case-control (366 cases, 469 controls)	“traditional” (inversely associated) “monotonous” (positively associated)	Oral Cancer	Marchioni et. al., 2007
Jakarta, Indonesia	Case-control (81 cases, 162 controls)	“preferred”, “chemical-related” and “traditional” (positively associated) “combination” (inversely associated)	Oral Cancer	Amtha et. al., 2009
Rio de Janeiro, Brazil	Case-control (210 cases, 251 controls)	“prudent” and “traditional” (inversely associated)	Oral and pharyngeal Cancer	Toledo et. al., 2010
Italy	Case-control (804 cases, 2080 controls)	“animal products” (inversely associated) “starch-rich”, vitamins and fiber” and “unsaturated fats” (positively associated)	Oral and pharyngeal Cancer	Edefonti et. al., 2010

5.1.4 Food Frequencies Questionnaire (FFQ)

There are some disadvantages in using FFQ as many details of dietary intake are not measured properly in terms of incomplete listings of all possible foods, errors in frequency and usual serving size estimation yielding inaccurate estimates of the average food intake. This happens when longer food listings in FFQ tend to overestimate while shorter lists may underestimate food consumption (Thompson & Subar, 2008). However, the FFQ used in this study was validated by choosing the most frequently used portion size as well as including only the typical foods that is usually consumed by the population of Malaysia. Although FFQ is not appropriate for estimating the nutrient intakes of food groups, it is reliable in terms of determining the habitual intake or a food pattern in large epidemiological case-control studies (Rossing *et. al.*, 1989 and Lee & Nieman, 1996). FFQ can also be considered as one of the most important measurement tools in terms of nutritional/dietary data collection and seem to provide a cost-effective method and is less invasive while reducing the duration of a research.

5.2 Hypotheses

Based on the findings of this study, the following hypothesis is rejected:

- a) There is no association between consumption of food items/group with oral cancer risk.

whereas the following hypothesis is accepted:

- b) There is no association between intakes of β -carotene with the risk of oral cancer.

5.3 Socio-demographic characteristics and oral cancer risk

In this study, all case and control subjects were randomly sampled from the database where matching was done for age (± 5 years), gender and ethnicity to minimize selection bias. Inclusion and exclusion criteria set for this study were also adopted according to the case-control studies that have been conducted in many other countries (Marchioni *et. al.*, 2007; Amtha *et. al.*, 2009 and Toledo *et. al.*, 2010).

5.3.1 Age and gender

Globally, it has been well documented that the development of oral cancer increases with age and the majority of cases occurs among those aged fifty years and over. The prevalence of oral cancer is more common among men than women (Warnakulasuriya, 2009). In the present study, the same distribution of oral cancer was observed with the highest number of oral cancer cases among those aged 50 years and above and male was being represented slightly more than female. The reported gender difference is probably due to heavier indulgence in the risk habits of tobacco smoking and alcohol consumption among men as compared to the women (Warnakulasuriya, 2010).

5.3.2 Association of risk habits and oral cancer

Worldwide, the three well known attributable risk factors for development of oral cancer were still the habits of tobacco smoking, alcohol consumption and betel quid chewing (Rao *et. al.*, 1994; Vecchia *et. al.*, 1997; Filho, 2002 and Yen *et. al.*, 2008). These

habits are practised in Malaysia where the findings from this study were concurrent for the above mentioned risk factors, resulting with an increased risk of about three-fold in smokers and drinkers whereas a two-fold increased risk was observed in chewers. The result is also consistent from findings in other countries where similar increased risk were observed associated with these factors (Dikshit & Kanhere, 2000; Zavras *et. al.*, 2001 and Stefani *et. al.*, 2008). Among the three major habits for cases and control groups that were investigated in this study, tobacco smoking was commonly the most practiced (43.1% cases and 17.6% controls) as compared to betel quid chewing (32.0% cases and 17.6% controls) and alcohol consumption (31.4% cases and 13.7% controls) respectively. Consumption of tobacco products has increased drastically due to mass production of cigarettes all over the world. This result may reflect an increased risk of oral cancer development in the population of Malaysia unless measures are undertaken to overcome this problem.

The mechanisms involved for the above mentioned risk has been well documented. The carcinogens produced from tobacco such as nitroso derivatives of nicotine, aromatic amines, benzene and heavy metals during smoking will cause metabolic changes in the body and higher toxicity to oral mucosa, thus leading to the development of oral cancer (Warnakulasuriya *et. al.*, 2005). Frequent consumption of ethanol (alcoholic beverages) may also increase the penetration of specific carcinogens such as N-nitrosodiethylamine by increasing permeability of the oral mucosa (Du *et. al.*, 2000). Moreover, long exposure related to alkaloids contained in areca nut, slaked lime and tobacco will tremendously enhance the pharmacological effects, resulting in oral submucous fibrosis and subsequently leading to oral cancer (Kumar & Zain, 2004).

This is also evident from the fact that joint effects of tobacco smoking and alcohol drinking will synergistically have multiplicative risk of oral cancer (Takezaki *et. al.*, 1996; Vecchia *et. al.*, 1997 and Moreno-Lopez *et. al.*, 2000). A study on tobacco quid chewing and “bidi” smoking has shown a significant association with oral cancer (Rao & Desai, 1998). At present, this study did not include the consumption type and duration of habit. Therefore, further research is advocated on the dose-response relationship which will provide better evidence in terms of indentifying and assessing the risk of oral cancer. This is because different types of tobacco use in cigarettes from different countries vary in terms of the prepared ingredients as well as the duration of the exposure to these habits is considered to be an important factor as it plays a major role in influencing oral cancer risk.

5.4 Dietary Intake

Studies on diet and oral cancer have long been investigated over the past decades (Vecchia *et. al.*, 1991). This include publications and reviews on several epidemiological studies between dietary factors with risk of oral cancer (Riboli & Norat, 2003). In most of the diet or nutrition studies, the most consistent findings were still the intake of fruits and/or vegetables that seems to confer protective effect on oral cancer (Vecchia *et. al.*, 2001). More than 50% of the observed results showed that consumption of fruits and vegetables or at least one type of fruit or vegetable confer protection for oral cancer (Franceschi *et. al.*, 1991; Levi *et. al.*, 1998 and Garavello *et. al.*, 2008).

The complexity of human diet as well as interpretation of correlation between diet and oral cancer continues to present a challenge in decades to come. This is because the

diversity of food combinations may lead to competition, antagonism or alteration in nutrient bioavailability (Amtha *et. al.*, 2009). Poor dietary practices, lack of physical activities, obesity and nutritional deficiencies have also been linked to the risk of developing oral cancer (Pavia *et. al.*, 2006 and Petti, 2008).

The possibilities of dealing with the complexity of inter-correlations and multi colinearity that exists between foods and nutrient is the use of pattern analysis. This approach uses correlations between food and nutrient intake to describe a general diet pattern that may be used as a reference of a particular disease. This method is applicable and of value if the effect of diet is not mediated or attributed by a single or two specific food items/nutrients but perhaps by the combination of food groups that operates interactively. In this present study, dietary pattern of all subjects were identified using principal component analysis and were subjected for statistical analysis to determine the risk of developing oral cancer.

5.4.1 Reliability of food groups/items

Although reliability test shows a poor consistency among the food items mentioned such as in starch, beverage and dairy groups, inclusion of these food groups for further analysis is important as these food are mostly consume and commonly found among the population of Malaysia. For instance, food item such as rice under the starch group is considered a staple food in Malaysia. Likewise, low reliability value, especially in the beverage group could also be due to the less representation of the food items as only carbonated drinks, coffee and tea were listed for the analysis. Moreover, Cronbach alpha

value is very sensitive to the number of items that were analyzed which may result in poor readings for this type of food groups.

5.4.2 Factor Analysis of Dietary Pattern

Factor analysis usually involves subjective or arbitrary decision that may have impact on both results and interpretation as the selection and grouping of foods for analysis, the method of rotation and the manner in which food groups were labelled, depend heavily on the researcher (Hirose *et. al.*, 2007). This multivariate method may also represent an alternative approach to the evaluation of single nutrients, since identification of patterns may allow one to examine the effects of the diet as a whole and also to describe association with disease. The patterns identified can act as a co-variable to determine whether the effect of a specific nutrient is independent of the dietary pattern. Though factor analysis is not commonly applied for dietary studies, it appears to be the most promising approach for the investigation of oral cancer with reference to the diet or combination of many dietary variables.

In the present study, factor analysis using Kaiser Criterion will have all 9 food groups extracted into 3 main components where Eigenvalue is more than 1. Since there are too many different food groups that fall under the same dietary component, it was then suggested to extract one more food component for further investigation to minimize cross loading of the food groups. As factor analysis was used for data exploration, it would also be worthwhile to retain 4 food components instead when a slight bend was observed at the third and fourth component respectively from the resulting scree plot (Pallant, 2005).

In this study, the grouping of food was based on the similarity of nutrients in terms of the major contribution from the food itself. Each of the food items listed in the group measured the same underlying factor such as in fruits and vegetables where the major nutrient contributor is vitamins or minerals. So, even though the loading of dairy foods is higher under the fruits and vegetables component, but considering the major nutrient contributor of dairy can be most accurately defined as ‘protein’ instead of vitamins, therefore, the inclusion of dairy in meat/by-products and fermented/salted food group was more nutritionally sound as both foods shared the same contributing nutrient. Other examples of food grouping were also based on the processing of the foods such as in processed and snack food items where they were characterized by the usage of high temperature and/or inclusion of chemical related products. Furthermore, all the food items were grouped into four different patterns by the similarity of composition and nutrient value based on previous studies (Marchioni *et. al.*, 2007; Amtha *et. al.*, 2009 and Toledo, *et. al.* 2010)

Interestingly, in this study, the reliability results of the four dietary patterns after undergoing factor analysis were moderately consistent, and possibly suggest stronger correlation among the different food grouping as a food component (factor) and thus provide meaningful data interpretation. In addition, the four retained food factors also explained more than half of the total variance from the study sample, thus it was useful for nutritional epidemiological research. If the patterns fail to explain much of the variance in the food intake as a whole, it could possibly mean that the patterns would not explain much of the variance in a single food or nutrient either (Schulze *et. al.*, 2001).

Results from dietary patterns that were analyzed through factor analysis yield the pattern of diet retained from the population. It is not appropriate to establish the relationship between nutrients and disease since this analysis is not specified for that purpose. Study on pattern analysis is useful when the traditional approach, which focused on specific food types, identifies only single food association and risk of disease. However, it is also widely known that individuals consume a variety of foods based on their food preferences/choices which are also influenced by various factors such as cultural differences, social status and demographic profiles.

5.4.3 Dietary pattern and risk of oral cancer

Little is known about the association between dietary patterns and risk of oral cancer when the supporting evidence is weak. The effects of individual food component as well as trace elements on carcinogenesis still remain unclear at present. This is because the issue of diet is rather complex as it not only affects the consumption of a single food item but also the interactions from other food types as well as the utilization, metabolism and digestion of these foods in the body. Dietary pattern studies may also be very useful to establish possible links and developing nutrition intervention-based programmes in Malaysia for the benefit of the community.

Since dietary patterns were extracted from the data obtained within the OCRCC-MOCDTBS database, the results cannot be generalized for the different multicultural dietary habits in other population. Though there are no studies yet on dietary pattern in Malaysia using factor analysis, the pattern observed in this study will provide an indication

of the most common foods consumed. Based on the findings and as a start for the Malaysian population, intervention guidelines could be issued with more emphasis on dietary patterns that are either health promoting or damaging to the population.

According to the adjusted risk estimates, the dietary pattern identified as “modern” which was characterized by consumption of processed and snacks foods was not associated with risk of oral cancer although it represented with the greatest communalities in this study. This dietary pattern of processed foods has much in common with other variables taken as a group and was loaded in the same component labelled as “modern”. The reason being that the various food preparation method such as freeze dried, deep fried, baked, roasted and frozen foods are easily available and on high demand where individuals on the go will usually consume pre-packed or fast foods (e.g. burger, pizza, sandwich, etc) to save time. In both univariate and multivariate analyses, the results obtained from this study were consistent with the study done by Marchioni *et. al.*, (2007) where there was no increase risk observed for higher intake of this food pattern. On the other hand, other researchers have found that the consumption of this food group was positively associated with oral cancer risk (Toporcov *et. al.*, 2004 and Amtha *et. al.*, 2009). Despite recommendations for limiting intake of such foods due to high level of fats that produce Polycyclic Aromatic Hydrocarbons (PAHs) under high temperature and during meat curing (e.g. bacon, sausages, etc), the results on intake of this component remained contradictory. The lack of increased risk found in the present study could be due to the fact that this component combined food items which were previously found to increase risk such as processed meat, bacon and sausages together with food items that is low in fats and have not been found to increase cancer risk such as traditional Malaysian “kuih” or biscuits, thus giving opposite

effects. Three earlier studies which were carried out by Macfarlane *et. al.*, (1995) also found no consistent effect on oral cancer risk with intake of macronutrients (fat, protein, carbohydrate) after controlling for total calories.

The intake of the second pattern group referred to as “prudent” was characterized by intake of fruits and vegetables, and found to confer protection of up to 47% before adjusting for confounding factors of tobacco smoking, alcohol consumption and betel quid chewing in multivariate analysis. However, after adjusting for the above mentioned confounding factors, high intake of fruits and vegetables does not seem to provide protective effect in reducing oral cancer risk. Similarly, in univariate analysis, no significant difference was observed for the consumption in the highest tertile for this dietary pattern. Furthermore, factor analysis indicates that a single factor alone may not account for disease causation as other factors from the same group will interact interchangeably. In contrast, results obtained from some studies noted that higher consumption of fruits may confer more protective effects than in vegetables (Vecchia *et. al.*, 1991; Petridou *et. al.*, 2002 and Riboli & Norat, 2003). This could partly be due to the differences in terms of nutritional content (chemical composition) in fruits and vegetables as well as the intakes of this food patterns that rely much heavily on the individual dietary habits. This finding was also supported by other research study where the apparent protection from fruits and vegetable could be affected by overlapping mechanisms of action which includes induction of detoxification enzyme, antioxidant effect, and dilution of carcinogens or alteration of hormone metabolism, thus providing confusing evidence (Vecchia *et. al.*, 2001). Fruits and vegetables have always been proven to confer protective effects in many types of cancers due to the constituent of fiber from the plant itself which is

known to bind carcinogens and limiting the contact time with the upper digestive tract (Zheng *et. al.*, 1993; Steinmetz & Potter, 1996 and Soler *et. al.*, 2001). It is also evident from the fact that fruits and vegetables do contain various phytochemicals and micronutrients that seem to provide beneficial health effects. However, habitual cooking of vegetables under high temperature or in abundance of water (soup-based) also tend to destroy, dissolve or dilute many of these water/fat soluble vitamins resulting in depletion of the protective constituent that may be considered helpful (Amtha *et. al.*, 2009).

In both univariate and multivariate analyses, the consumption in the highest tertile of the third dietary pattern, termed as “combination” which is categorized by dairy, meat/byproduct and fermented/salted food was significantly associated with the risk of oral cancer. A plausible explanation of the contribution of this dietary pattern to oral cancer is due to the technique of food preparation where broiling, frying and barbecuing protein-rich high-fat foods such as red meats at high temperatures can cause formation of heterocyclic amines (HAs) (Grosvenor & Smolin, 2002). The chemical component (carcinogenic agent) found in these food groups has been implicated in the initiation of cancer due to the interaction of amines and nitrite or nitrous oxide where it usually occurs in cured meat and salt-dried fish (Kreimer *et. al.*, 2006). The findings in this study was supported by other studies which provide evidence linking high meat and dairy food consumption to increased risk of oral cancer (Levi *et. al.*, 1998; Gallus *et. al.*, 2006 and Franceschi *et. al.*, 1999). Saturated fatty acid foods that are high in glycaemia indices promote inflammation that has been linked to some serious disease such as cancer. Advanced glycated end-products (AGEs) are created when sugars bind with protein rich foods such as dairy products and thus raise the body production of reactive agent that is damaging to health (Yap, 2011).

Contradictory findings were observed from a study done in Indonesia by Amtha *et. al.*, (2009) where an inverse association on oral cancer risk was observed for consumption in meat and dairy products. Even though the consumption of protein food is needed for the body in cell building and defence (immune) mechanisms, the method of food preparation has to be taken into consideration as influence or introduction of carcinogenic agent in the body could possibly lead to the development of oral cancer.

Similarly, the fourth dietary component labeled as “traditional” which was characterized by intake of starches (staple food for Malaysians such as rice, bread and mee) and beverages (carbonated drinks, coffee and tea) was associated with increased risk of oral cancer. Consumption in the highest tertile of high calories/sugar foods could possibly lead to many other chronic diseases such as diabetes if individuals continue to practice sedentary lifestyle. Chronic diseases such as cancer could result from prolonged consumption of high amount of acidic foods such as refined carbohydrates and beverages such as in soft/carbonated drinks and coffee. The body maintains its alkalinity at a constant pH of 7.4 and consistently adapts to the changes of acid/base balance though the foods consumed (Yap, 2011). A study by Levi *et. al.*, (2000) also found an association between refined grains consumption with the risk of oral cavity cancers. High intake of carbohydrate such as rice has also been shown to moderately increase the risk of oral cancer from a study conducted in Beijing (Zheng *et. al.*, 1993). However, contradictory findings were reported on coffee and tea consumption which showed decrease risk of oral cancer by Tavani and Vecchia, (2004) possibly due to the antioxidant properties found in the coffee beans or in the tea leaves. Similarly, studies conducted in Brazil by Toledo *et. al.*, (2010) and Marchioni *et. al.*, (2007) also found an inverse association for “traditional” pattern

consumption. The oral cancer risk associated with this pattern remains unclear as not many studies have been done and it could possibly depend on the type of carbohydrate consumed, how glucose is metabolized in the body and its influence on short chain fatty acid production obtained from all the food ingested.

In summary, consumption in the highest tertile of both ‘combination’ and ‘traditional’ pattern display a significant increased risk of developing oral cancer. On the other hand, consumption of “prudent” pattern suggests a non-significant reduced risk after adjusting for confounding factors. However, consumption for ‘modern’ pattern does not have much effect in terms of reducing oral cancer risk as it was not statistically significant.

The mechanisms involved in the prevention of cancer development by dietary regimens are believed to be mediated by several factors such as the effect of dietary compounds and detoxifying enzymes from the foods consumed. Therefore, diet modification for oral cancer prevention in Malaysia should include low consumption of foods that are highly carcinogenic, as well as increasing intake of foods that have high detoxification value (Meurman, 2010). Further research is suggested on the effects of food composition in terms of macro and micro nutrients and adjusting the calorie intake (Northstone *et. al.*, 2008) to provide the supporting link of developing nutritional programmes to reduce oral cancer risk in Malaysia.

5.5 Intake of β -carotene and oral cancer risk

No statistically significant association between high intake of carotene and risk of oral cancer was found in this study. In contrast, many epidemiological studies suggest that low dietary intake and low plasma concentration of antioxidant vitamin such as β -carotene are associated with increased risk of cancer (Steinmetz & Potter, 1996; Vecchia *et. al.*, 2001 and Khaodhiar & Blackburn, 2006). Vegetables and fruits have a rich resource of nutrients with validated anti-oxidant properties such as β -carotene, Vitamin C and α -tocopherol that occurs naturally and are potent regulators of cellular activities and have significant impact on oral carcinogenesis (Enwonwu & Meeks, 1995). It was also hypothesized that β -carotene may help in preventing tissue damage by trapping organic free radicals and/or deactivating excited oxygen molecules.

The contrasting finding in this present study could be due to the highly skewed distribution of β -carotene consumption of respondents towards low intake in their daily diet. More than 85% of all the case and control subjects do not meet the daily Recommended Nutrient Intake (RNI) as provided by the national guideline. This shows that the Malaysian population does not consume as much of vitamins supplementation to significantly produce acceptable results. It is also interesting to note that the study of 'prudent' food pattern as mentioned earlier, which comprise of fruits and vegetables (rich sources of β -carotene) also showed no significant association with oral cancer risk. Petridou *et. al.* (2002) and Maserejian *et. al.* (2006) also reported no significant association between the intake of carotene, β -carotene-rich fruits and vegetables with oral cancer risk.

The role of β -carotene in oral cancer development is conflicting where no clear evidence is found as reported in a review study by Chainani-Wu (2002). Studies from a single vitamin (nutrient) was not found to have any protective effect on oral cancer risk but multiple vitamin studied synergistically has been found to provide reduced risk of oral cancer (Lucenteforte *et. al.*, 2009). Some review studies are conducted in different population and the average intake of β -carotene in that population may differ quite substantially resulting in contradicting results obtained for the population of Malaysia. Other interpretation of observation in carotenoids study could arise from the lack of availability due to inadequate β -carotene food composition table or other dietary factors that are correlated with β -carotene intake (Mayne, 1996).

An important aspect worth considering in terms of β -carotene intake is the method of food preparation. Association between β -carotene with oral cancer risk is not attributed only to the nutrient from the food content itself, but is also influenced by the method of preparation of foods. Fruits and vegetables are easily oxidized when exposed in the air or cooking under heat for a long period of time resulting in depletion of all vitamins and minerals. Foods that are eaten raw are believed to have higher level of antioxidant properties as the constituent of chemical component still remain intact in the plant itself as compared to foods that have been cooked because these vitamins may have been destroyed during the process of cooking. It is not known how the effects of β -carotene consumption could possibly help to reduce oral cancer risk. Therefore, future research on human intervention trial via biochemical assessment is very much needed to establish a proper link between micronutrient intake and oral cancer risk.

Chapter 6

Conclusions and Recommendations

This chapter presents the conclusion of this study, based on the results obtained in Chapter 4. Suggestions are forwarded for future studies so that greater understanding on dietary factors/groups involved may possibly help to reduce the development of oral cancer in Malaysia.

6.1 Conclusion

It is well documented worldwide that the main habitual risk factors for oral cancer development is tobacco smoking, alcohol consumption and betel quid chewing. Nevertheless, the role of diet and oral cancer has also been shown by epidemiological studies to play an important role in enhancing or reducing the risk of oral cancer. Thus, there is a need to continue research on diet or nutrition in order to obtain baseline data for oral cancer in Malaysia. Additionally, conflicting evidence continue to arise due to individual food preferences/preparation which makes it difficult to properly establish an association as there are no single dietary factor that explains any interaction between consumption of food and oral cancer (Meurman, 2010 and Boyle *et. al.*, 2008). Therefore, this study was conducted to investigate the dietary pattern as well as β -carotene intake to determine the correlation between these dietary risk factors and its association with oral cancer.

6.1.1 Risk habits of oral cancer in Malaysia

The main attribute of oral cancer risk in Malaysia is due to tobacco smoking, alcohol consumption and betel quid chewing. This is evident from the finding that smokers have increased risk of developing oral cancer by 3.5 times, drinkers by 2.9 times and chewers by 2.2 times as compared to those who do not. Moreover, the increasing trend of oral cancer development is also supported by high prevalence of case as compared to control subjects for habitual smoking (43.1%), drinking (31.4%) and chewing (32.0%) respectively. However, measurement of lifetime exposure to the risk habits for both dose and duration will provide better understanding and assessment towards oral cancer development and this can be the subject for future research.

6.1.2 Dietary patterns

This study provides evidence of the meaningful association between dietary patterns and oral cancer through factor analysis. Consumption in the highest tertile of the 'traditional' pattern accounted for 9.2% of the total variance, which was characterized by intakes of starches and beverages, showed twice the risk of developing oral cancer. Similarly, the highest tertile by intakes of 'combination' pattern accounted for 11.8% of the total variance, which was loaded by dairy, fermented/salted and meat/by-products may induce thrice the risk of oral cancer. In contrast, 'prudent' pattern consumption of fruits and vegetables which accounted for 13.5% of the total variance was not associated with oral cancer risk after adjusting for confounding factors. No significant association was observed

for “modern” pattern which was loaded with processed foods and snacks, the most important component in this study, which accounted for 34.8% of the total variance.

6.1.3 Intake of β -carotene

No significant association was observed between high intake of β -carotene (OR 0.83, 95%CI: 0.42-1.66, $p>0.05$) with the risk of oral cancer. Moreover, findings from the ‘prudent’ dietary pattern also showed no significant association, thus further supporting this finding.

6.2 Future recommendations

There are several possible directions for future research that could be suggested to help widen the scope of food nutrition analysis, improving the data for oral cancer, especially on dietary pattern and their association with oral cancer in Malaysia. The following suggestion includes:-

- a) The Malaysian population consist of diverse ethnicities. Hence, future research studies on diet should include other ethnic groups to improve the generalization of the findings. Therefore, sample location will have to be broaden to include other ethnic groups as an entity and that the sample size to be increased and duration of the data collection to be extended.

- b) In terms of methodological concern, attention and consideration should be given in selecting controls subjects as hospital-based controls can bias the diet-environment interactions. Therefore, it is recommended that more community controls be used in future studies.
- c) Further research on β -carotene via biochemical analysis of nutrients from human plasma or serum could be more useful in terms of determining the actual nutritional status of an individual.
- d) The failure to demonstrate any association between intake of β -carotene and oral cancer risk is partly due to a lack of statistical power. Thus, a larger sample size is required for future design on case control study using population based controls with a much better spread of β -carotene consumption.